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THE HEREDITARY AND ENVIRONMENTAL PORTIONS OF THE VARIANCE IN WEANING WEIGHTS OF POLAND-CHINA PIGS[1]

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Received, April 1, 1937

MATERIAL AND METHODS

IN 1930 a study of the effects of inbreeding on Poland-China pigs was begun at the Iowa Agricultural Experiment Station. The foundation animals, 27 sows and three boars, were purchased from various breeders. Most of them were only about six months old when purchased but a few were older gilts which had already been bred. In this way some offspring of three other boars were also added to the herd. Since the summer of 1930 no breeding stock has been purchased, all replacements being selected each spring from the more promising individuals raised. The first selections were made at weaning time and were based on the individuality of the pig and the production records of its dam. At examinations later in the summer or in the fall some of the pigs selected at first were rejected and were not actually used for breeding.

Generally four boars were used each season. Each boar was mated to a representative group of sows in so far as concerned age, production, type-score and relationship to their mates. Thus from each boar there came some closely inbred and some outbred pigs. Fall farrowing duplicated spring farrowing as nearly as possible except that few gilts farrowed in the fall following their first litters. Only 12 of the foundation animals were inbred (pedigrees being traced to 1923 as a base date), and they were less than four percent inbred. The highest relationship between any two of these 44 animals was 32 percent in the case of non-litter mates and 51 percent between three individuals which were litter mates. The average inbreeding of the herd has been rising slowly but even in 1935 (the last year included in this study) the average inbreeding of all pigs weaned (11.8 percent) was less than that produced by mating half brother and sister in a random breeding population. The average inbreeding (8.5 percent) of the 1633 pigs weaned in the entire six years was only a little above that (6.2 percent) which would result from mating ordinary first cousins. Assortive mating was expressly avoided, care being taken to mate each boar to a typical sample of sows.

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All animals in the breeding herd were fed good practical rations. Except for the breeding system and the detailed record taking, the general management to which these pigs were subjected was quite similar to that practiced in good commercial herds. Each pig was weighed at birth and when 60 days old was weaned, weighed and scored. The present study is of the weaning weights of 1633 pigs farrowed in 271 litters out of 111 sows mated to 20 boars in the six-year period from 1930 through 1935. The average number of pigs weaned per litter was 6.03 and the average weaning weight per pig was 40.1 pounds.

The data were analyzed mainly by the method of analysis of variance (FISHER 1932). The observations are classified on the basis of certain criteria which it is supposed may be significant causes of variation and the total variance is analyzed into its component parts. The relation between the total variance and the variance left after eliminating that between classes can be expressed either as a correlation between the observations within a class, or as that fraction of the variance caused by the factor or complex of factors, which affect all members of a class alike. Computations were greatly facilitated by punching the data on cards for use in a Hollerith tabulating machine.

ANALYSIS OF DATA[3]

The influence of sex

When the weaning weights were grouped according to the sex of the pigs the total variance was reduced 0.2 percent. This effect was on the borderline of statistical significance but was too small to be of much, if any, practical importance and hence was neglected in the remainder of the study. The males averaged 0.9 pounds heavier than the females.

The influence of age of dam and season

Table 1 shows the number and average weights of pigs weaned by sows of the several ages. Nearly all sows saved for breeding were farrowed in March and April and a few in May. Spring farrowing was in those months with an occasional late litter in June. Fall farrowing was mostly in September with a few litters in October and August. The difference between spring and fall pigs, $5.6 \pm .51$ pounds, is highly significant statistically and seems large enough to be important economically. Table 2 shows the analysis of variance in weaning weight on the basis of season and age of dam. Seven percent of the original variance disappears when the seasonal effect is deducted but this probably includes some variance which comes from age of dam.

[3] Tables giving analyses of variance due to size of litters weaned, years, litter, dam and sire have been placed on file in the "Genetics" editorial office and may be consulted by anyone interested.

The variance due to the age of the dam cannot be isolated completely from the difference between farrowing seasons, since no age is represented in both seasons. The mean square for differences between ages within season based on the eight degrees of freedom, was significantly larger than

TABLE 1

Weaning weight of pigs classed by season of birth and by age of dam.

APPROXIMATE AGE OF DAM (YRS.)	NUMBER OF PIGS		AVERAGE WEIGHT (LBS.)	
	SPRING	FALL	SPRING	FALL
1.0	604		35.7	
1.5		83		39.8
2.0	362		41.1	
2.5		201		45.2
3.0	172		42.3	
3.5		86		47.1
4.0	64		42.7	
4.5		22		43.7
5.0	23		40.8	
5.5		16		43.1
Total	1225	408	38.7±.25*	44.3±.44

* In this article the figure following the ± sign is the standard error instead of the probable error.

TABLE 2

Analysis of variance in weaning weight due to season and age of dam.

SOURCE OF VARIANCE	D/f	SUM OF SQUARES	MEAN SQUARE
Total	1632	138,518	84.9
Between seasons	1	9,787	9,787.5
Within season	1631	128,731	78.9
Between ages within season	8	13,275	1,659.3
Within age and season	1623	115,456	71.1
Between litters born in the same season from dams of the same age	261	52,216	200.1

$$\frac{84.9-78.9}{84.9}=.070 \qquad \frac{84.9-71.1}{84.9}=.162$$

the mean square between litters weaned by dams of the same age and in the same season. Thus there remained some real differences due to the age of dams, even after the seasonal effect had been eliminated. The combined effects of farrowing season and of age of dam account for about 16 percent of the original variance (table 2).

Examination of figure I suggests that the small average weight of the pigs from immature dams may have been the main source of the variance which disappeared with the seasonal difference and with the age differences among dams farrowing in the same season. To test this suggestion all litters from dams farrowing at about one year and at about 1.5 years were

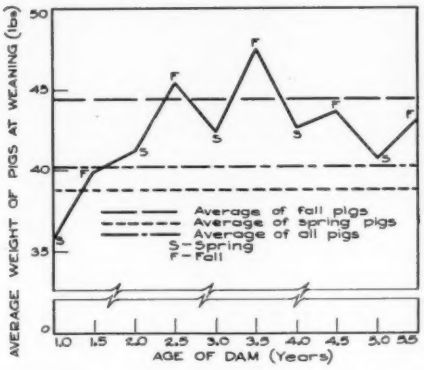

FIGURE 1. Average weights of pigs weaned by dams at different ages.

removed and the remaining data were re-analyzed as shown in table 3. An important seasonal effect still remains. However the variance due to the ages of dams within their respective seasons has been reduced even below that shown in the last line of table 2. That is to say, there was little if any influence of age of dam on the weaning weights of pigs after the sows are as much as 24 months old. However none of the sows in this experiment had yet passed 5.5 years. Hence the data tell nothing about age-effects which might possibly occur at older ages than that.

TABLE 3

Analysis of variance due to age of dam and season after eliminating litters from young sows.

SOURCE OF VARIANCE	D/f	SUM OF SQUARES	MEAN SQUARE
Between age and season	7	3,937	562
Between seasons	1	3,279	3,279
Between ages of dams free of season	6	658	110

A factor which might possibly contribute something to the apparent effect of age of dam is the culling of some of the less productive sows. This was done after each farrowing season, but more particularly after the spring farrowing. So far as such culling was effective, one would expect a little less variability in the pigs from groups of sows of older ages and a

slight superiority in the pigs weaned in the fall over pigs weaned in the spring. The distinct seasonal difference might possibly be due to factors of climate. Perhaps the sows were in better physical or nutritional conditions in the fall and as a consequence may have produced more milk. Or temperature or other weather factors or supplementary pasture may have had a still more direct effect on the growth rates of the pigs.

Influence of size of litter weaned

There was some variation in the average weaning weight of pigs from litter size to litter size. Figure 2 shows a slight tendency for this weight to decrease (at least in litters larger than 3) with an increase in the number of

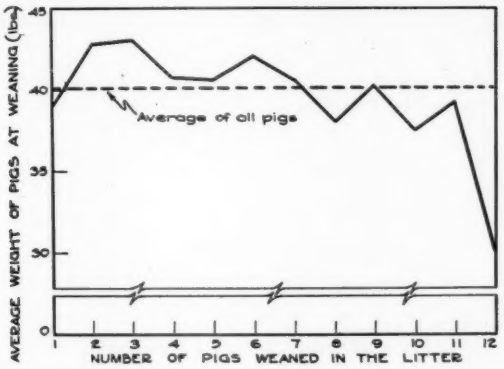

FIGURE 2. Average weaning weights of pigs classified according to number of pigs in the litter at weaning time.

pigs weaned in the litter. AXELSSON (1934) found a similar relationship in data from Swedish herds, using three-week weights.

The variance between litter sizes, when compared with that between litters of the same size has a probability of one chance in twenty of being due to errors of sampling. If all pigs had been weaned in litters of the same size, the total variance would have been reduced about three percent. It appears that the size of the litter weaned has only a small effect on the weight of the pigs therein.

Influence of year

Since these weights were taken over a period of six years, the question arose as to whether there had been any difference in the weaning weight of the pigs from one year to another. There are data to show that the variance between years was of doubtful significance when compared with the variance between litters weaned in the same year. The reduction in

variance amounted to about one and one-half percent. Apparently the feeding and management from year to year have been quite uniform and there has been no marked change in the genetic level of the herd for weaning weight. There is no reason to believe that compensating changes occurred in these factors. Such small differences as actually existed between the yearly average weaning weights may very well have come from the larger proportion of young dams in the first two years or from small yearly variations in the proportion of fall to spring pigs.

TABLE 4

Average weaning weight of pigs in litters of different sizes.

LITTER SIZES	NO. OF LITTERS	NUMBER OF PIGS			AVERAGE WEIGHT (LBS.)		
		SPRING	FALL	TOTAL	SPRING	FALL	TOTAL
1	8	5	3	8	38.8	39.3	39.0
2	13	18	8	26	42.1	44.0	42.7
3	18	39	15	54	41.1	48.2	43.1
4	26	84	20	104	39.2	47.4	40.8
5	31	120	35	155	38.9	46.8	40.7
6	48	228	60	288	41.8	43.0	42.0
7	55	259	126	385	38.6	44.8	40.6
8	50	328	72	400	36.9	42.6	37.9
9	13	90	27	117	38.3	46.4	40.2
10	4	20	20	40	33.2	42.0	37.6
11	4	22	22	44	36.0	42.5	39.2
12	1	12	—	12	30.2	—	30.2
All	271	1225	408	1633	38.7	44.3	40.1

The genetic portion of the variance

No estimate of the amount of genetic variance in a population can be made without considering in some form or other the comparative likeness between relatives. Yet the observed correlations between relatives, especially among farm animals, may be enhanced or diminished by the influence of environment, according to the importance of environmental variations in modifying the character and according to the correlations between the environments under which different kinds of relatives are raised. Moreover some of the gene pairs may exhibit some degree of dominance and some genes may interact in non-additive ways, that is, the effects of some gene combinations may be more (or less) than the sum of the average effects of those genes considered separately. These complications prevent obtaining precise answers to the question of how important each kind of genetic variance is. Yet the correlations between various kinds of relatives may indicate roughly what answers are reasonable. The results of doing that are presented next.

The correlation between litter-mates

In these data the variance among pigs weaned in the same litters is about 45 percent less than the variance in the whole population. This corresponds to a correlation coefficient of .45 between litter mates and is highly significant. The standard error is about .05. This correlation between litter mates is a sum of four different kinds of things which might make litter mates alike, as follows (WRIGHT 1935):

1. About half of the additive genetic fraction of the variance.
2. About one-fourth of the fraction of the variance which comes from dominance deviations.
3. About one-fourth of the fraction of the variance which comes from non-additive interactions of genes in different allelic pairs.
4. The environmental fraction of the variance multiplied by the correlation between the environments of litter mates. This last correlation may well be high and contribute much to the likeness between litter mates, since they go through their embryology in the same uterus and are nursed by the same sow. Also they are perfect contemporaries in their exposure to variations in weather, nutritional conditions, parasites and bacterial infections.

Since the correlation between litter mates is so complex in its origin and probably includes so much of environmental origin, it throws little light by itself on the question of how much of the variance is "hereditary," even if we lump together under that one term all three of the genetic kinds of variance listed above.

Correlation between weaning weights of dam and offspring

Frequently the most useful measure for determining the genetic portion of the variance in a character is a direct correlation of that character between parent and offspring. This is especially apt to be the case in this problem where, because the data all come from the one herd under reasonably uniform management, there is reason to suppose that the non-genetic correlation between the pre-weaning environments of parent and offspring was not substantially different from zero.

Of the 111 sows represented in these data 85 had recorded weaning weights, the others having been purchased when grown or partly grown. These 85 sows were mated to a total of 16 boars to produce 1118 pigs in 184 litters. The gross correlation between the weaning weights of these sows and that of their pigs was +.0006 or essentially zero in the entire population. In order to free this correlation from the effects of the sire and of the age of dam, an analysis of covariance was carried out on the basis of sire and age of dam (table 5).

The correlation "within groups of dams of the same age mated to the same sire" (.058) is a measure of the likeness between the weaning weights

of dams and the weaning weights of their pigs when the dams are all of one age and mated to the same boar. However, the above correlation is based on an offspring variance reduced by removing the sire effect and the effect of the age of the dams. This makes the correlation higher than it would have been on the basis of the original variance. The correlation desired, therefore, is one in which due account has been given to these effects but is based on the original variance within the whole population. To return to the original base we multiply the correlation of .058 by the square root of the ratio of the reduced variance to the original variance. Thus $\sqrt{59.77/80.50} \times .058 = .050$, the desired correlation. No corresponding

TABLE 5

Analysis of covariance between weaning weights of dams and offspring.

SOURCE OF VARIANCE	D/f	DAM'S WEANING WEIGHT		PIG'S WEANING WEIGHT		COVARIANCE	
		SUM OF SQUARES	MEAN SQUARE	SUM OF SQUARES	MEAN SQUARE	SUM OF PRODUCTS	r
Total	1117	79205	70.9	89920	80.5	54	.0006
Between groups of dams mated to the same sire	15	16819	1121.3	4242	282.8	2455	.2907
Within groups of dams mated to the same sire	1102	62386	56.6	85677	77.8	−2402	−.0328
Between groups of dams of the same age, mated to the same sire	50	26179	523.6	22808	456.2	−5178	−.2119
Within groups of dams of the same age, mated to the same sire	1052	36207	34.4	62869	59.8	2776	.0582

correction was made for the reduction in the variance in dam's weaning weight since much of that arises from the repetition of the dam's weight for each pig she weaned and presumably would have affected the covariance by a proportional amount. So far as selection of the dams tended to keep those with small weaning weights from becoming dams, the observed parent-offspring correlation probably is a little smaller than it would have been in a population in which there was no selection at all for weaning weight. However the amount of this effect must have been small in the present case. This parent-offspring correlation is made up of one half of that hereditary variance which can be construed as resulting from additive gene effects and one quarter of the epistatic effects of genes in bringing about variations in the pigs' weaning weights. Doubling this correlation yields 10 percent as the portion of the variance which includes the additive genetic variance and half of the variance from non-additive gene interactions.

The correlation between maternal half-sibs

Analysis of variance due to dam shows that when those things common to maternal sibs are held constant, a reduction of 21 percent of the

original variance is effected. This correlation involves full sibs as well as maternal half sibs and may also be affected by the dam's age and by seasonal differences since, of course, not all dams weaned pigs at all ages or weaned the same proportion of fall pigs to spring pigs.

Correction for the inclusion of full sibs was made by using the correlation between litter mates in conjunction with the proportions of the maternal sib comparisons which were between full sibs and between half sibs. The corrected correlation between maternal half sibs was .114. This includes: (1) about one-fourth of the additive genetic fraction of the variance, (2) probably about one-sixteenth of the epistatic fraction, and (3) the environmental fraction multiplied by the correlation between the environments of maternal half-sibs. This environmental correlation presumably results largely from permanent differences in nursing abilities between dams but in part from some dams having only spring litters while others have both spring and fall litters. This correlation probably is too large to be ignored although it doubtless is much lower than that between litter mates since maternal half-sibs are not contemporaries.

Correlation between paternal half sibs

The variance in weaning weight between sires has been analyzed; the 4.7 percent reduction in variance corresponds to a correlation of .047 between pigs all having the same sire. It does not, however, take into account the fact that many of the comparisons within sires are comparisons between full sibs. Correcting for the effect of these full sib comparisons gave +.017 for the correlation between paternal half sibs unbiased by full sib comparisons.

Because there was almost no variance from general year-to-year changes, and because an effort was made to mate each boar to a representative group of sows and because many of the sires were used in more than one season it will not be far wrong to assume that none of this correlation arises from environmental correlations between paternal half sibs. The .017 therefore includes about one-fourth of the additive genetic fraction of the variance and probably about one-sixteenth of the epistatic fraction.

Combination of evidence from various relatives

The foregoing analyses may be summarized in the following equations, using for convenience symbols as follows:

h^2g^2 = the additively genetic fraction of the variance

h^2d^2 = that fraction of the variance which comes from dominance deviations from the additive scheme

h^2i^2 = that fraction of the variance which comes from non-additive interactions between genes of different allelic pairs

e^2 = that fraction of the variance which comes from differences in the environment

From litter mate correlations: $\frac{h^2g^2}{2}+\frac{h^2d^2}{4}+\frac{h^2i^2}{4}+r_{EE}e^2=.453$

From offspring-dam correlations: $\frac{h^2g^2}{2}+\frac{h^2i^2}{4}=.050$

From maternal half-sib correlations: $\frac{h^2g^2}{4}+\frac{h^2i^2}{16}+r_{EE'}e^2=.114$

From paternal half-sib correlations: $\frac{h^2g^2}{4}+\frac{h^2i^2}{16}=.017$

Comparing the paternal half-sib and the parent-offspring correlation it appears that h^2i^2 is .128 and h^2g^2 is about .036, and by comparing the correlations between the two kinds of half-sibs it appears that the portion of the variance due to permanent individual peculiarities of the dams is about .097. This last figure is doubtless somewhat lower than it really should be, since between maternal half-sib litters there are *always* differences in the age of dam and differences in the farrowing season are more frequent than among pairs of litters selected strictly at random. The ratio of h^2d^2 to h^2g^2 is typically about 1 to 2 according to FISHER (1918) or more nearly 1 to 4 according to WRIGHT (1931), the difference in the two views depending largely on whether complete dominance is the rule for minor genes as well as for major ones, the nature of the breeding system, the frequency of dominant and recessive genes and such facts not yet definitely ascertained for whole populations. These two ratios yield values of .018 or .009 respectively for h^2d^2. Use of the above values yields values of .401 to .399 for the correlation between litter mates due to their having been under nearly the same environments.

Now these computations rest on observations each with sampling errors of its own and these sampling errors are increased by multiplying and subtracting from each other the observed correlations to obtain values for h^2g^2, h^2d^2, etc. Moreover there is some doubt that the formulae used are entirely adequate for h^2i^2, even though WRIGHT (1935) has shown that the correlations between relatives so far as they rest on h^2i^2 cannot be less than the square of the correlation which would exist if all interactions between non-allelic genes were additive. In view of these uncertainties and sampling errors, the values obtained should be considered only as rough approximations. They are given in table 6 only to the nearest one percent.

Without pressing the validity of these estimates too far, it seems safe

to conclude that the heredity of the pig plays a minor part (something like a tenth to a quarter) in determining its weaning weight and that circumstances or environments which affect even litter mates differently play a major part (perhaps only a little less than one-half). Important contributions to the variance between litter mates are the causes which produce the extremely small pigs usually known as "runts." Although these runts are few in number, yet they contribute much to the variance within litters and make the distribution curve somewhat skewed. Their almost random distribution among litters makes it unlikely that any large proportion of them are due to the segregation of single genes with important sub-lethal effects. If they are caused by minor unnoticed diseases or parasitic infection, it is surprising that they should be scattered so nearly at random through the various litters. It is more plausible to suppose that many of them represent what might be called embryological or developmental accidents which are without a definite genetic basis and which have placed serious handicaps on the individuals to which they occurred. Also there may be differences in the productiveness of some of the mammae and, as pigs have some tendency to use a particular nipple most of the time, an initial handicap resulting from this might become progressively greater as the handicapped pig became less and less able to compete with its litter mates.

These particular data were all from one breed and the breeding system was beginning to bring a moderate degree of homogeneity to this herd by the time (1935) the latest litters were born. Probably the percentage of hereditary variance would have been slightly larger in a population made up of several breeds or of a highly heterogeneous herd of one breed. Also this herd was kept under a reasonably uniform system of feeding and management. Had it included sows and litters fed and managed in all sorts of diverse and extreme ways, the relative part played by "other" environment common to litter mates would certainly have been larger at the expense of many or all the other causes of variation. That is, these estimates apply to this particular population and caution should be observed in extending them to widely different populations, although there seems no positive reason for thinking that they would not be typical of reasonably well managed one-breed herds.

SUMMARY AND CONCLUSIONS

An analysis of the variance in 60-day weaning weights among 1633 Poland-China pigs in 271 litters in the early stages of an inbreeding experiment showed a correlation of .45 between litter mates but only .05 between parent and offspring, .02 between paternal half-sibs and .11 between maternal half-sibs. From this and certain other information is made an

estimate (table 6) of the relative importance of heredity and certain environmental factors in causing variations in weaning weight.

TABLE 6

The relative importance of hereditary and environmental sources of variance in the weaning weight of Poland-China pigs.

SOURCE OF VARIANCE		PERCENT OF TOTAL VARIANCE
Heredity of the pig		
Additive genetic effects	4	
Dominance deviations	1	
Non-additive interactions	13	
Sex	trace	
	—	18
Environment common to litter mates		
Permanent differences betwen dams in their abilities as nurses (perhaps in part dependent on the heredity of those dams)	10	
Age of dam and season of farrowing (much of this from the age effect shown in first litters)	16	
Number of pigs in the litter at weaning time	3	
Year-to-year changes in environment	1	
Other	10	
	—	40
Environment not common to litter mates		42
TOTAL		100

The sex difference was on the border-line of statistical significance, but was too small to be of practical importance.

Age of dam and season together accounted for about 16 percent of the total variance in weaning weight. A large portion of this was due to yearling sows farrowing their first litter in the spring.

Contrary to expectation, the size of the litter at weaning appeared to have only a small influence on the weaning weight of the pigs.

The figures are subject to a large sampling error but seem to indicate rather clearly that the hereditary portion of the variance is moderately small and that a considerable portion of the variance (about 42 percent in these data) comes from what might be called prenatal environmental and embryological accidents which affect litter mates differently.

REFERENCES

AXELSSON, J., 1935 Einige Resultate der Sauen-Leistungskontrolle in Malmöhus. Län. Züchtungskunde **9**: 23–36.

FISHER, R. A., 1918 The correlation between relatives on the supposition of Mendelian inheritance. Trans. Roy. Soc. Edinburgh. **52**: 399–433.

FISHER, R. A., 1932 Statistical methods for research workers. 4 th ed. XIII+307 pp. London and Edinburgh, Oliver and Boyd.

WRIGHT, SEWALL, 1931 Evolution in Mendelian populations. Genetics **16**: 97–159.

1935 The analysis of variance and the correlations between relatives with respect to deviations from an optimum. J. Genet. **30**: 243–256.

FREQUENCY OF SPONTANEOUS MUTATIONS IN CERTAIN STOCKS OF DROSOPHILA MELANOGASTER

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Received April 24, 1937

DURING the investigations dealing with various problems in which the frequency of X chromosome lethals was measured, an unusually high frequency of spontaneous lethals was noticed in the inbred Florida stock. This marked the beginning of an attempt to ascertain the rate of spontaneous mutability in various wild type stocks. The results of these studies will be reported in the present paper. A preliminary account was presented at the 1936 summer meeting of the Genetics Society of America (DEMEREC 1937a). At the same meeting PLOUGH and HOLTHAUSEN (1937) reported a high frequency of visible mutants in crosses where the Florida stock was used.

METHODS

The major part of the experiment deals with the determination of the rate of occurrence of spontaneous X chromosome lethals. Throughout this work a uniform procedure was used. Lethals were detected by the ClB method according to the following scheme:

(1) ClB/*ec ct v g* ♀ by + ♂
(2) F_1 ClB/+ ♀ by *ec ct v g* ♂
(3) Females from a culture without males were bred as follows: +(*l*)/*ec ct v g* by *y Hw*, In dl–49.

Flies for (1) were raised in $\frac{1}{4}$ pint milk bottles, for (2) in 25×95 mm vials and (3) in $\frac{1}{4}$ pint milk bottles. In all cases corn-meal, molasses, and agar food was used, brewers' yeast having been added to make it richer. The food contained about 0.10 percent of Moldex-A.

RESULTS

Results of experimental tests of fifteen wild type stocks are summarized in table 1. These stocks, obtained from various laboratories, came from places so widely separated geographically that they could be only distantly related. The data show that three of them, Florida-inbred; Wooster, O.; and Formosa, Japan, have a significantly higher mutability rate than the other twelve stocks tested. Comparing the high mutability stocks with the low mutability Oregon–R, it was found that the Formosa stock has a mutability rate about five times as high as that of the Oregon–R, while Wooster showed a mutability rate about ten times and Florida almost twenty times as high as that of the Oregon stock.

TABLE 1

Frequency of spontaneous lethals in X chromosomes of various wild-type stocks.

STOCK	RECEIVED FROM	NUMBER OF X CHROMOSOMES	NUMBER OF LETHALS	PERCENT LETHALS
Florida-inbred	Columbia University, 1928	2108	23	1.09±0.15
Wooster, O.	Spencer, June 1936	1266	8	0.63±0.15
Formosa, Japan	Zool. Inst. Kyoto, Aug. 1936	2054	8	0.39±0.09
Oregon-R	Cal. Inst. Tech. (?)	3049	2	0.066±0.03
Swedish-b	Cal. Inst. Tech., April 1936	1627	3	0.18±0.07
California-c	Cal. Inst. Tech., June 1936	708	2	0.28±0.13
Huntsville, Tex.	Univ. of Texas, June 1936	938	—	—
Urbana, Ill.	Univ. of Illinois, July 1936	1016	1	
Canton, O.	Amherst Col., June 1936	922	—	—
Amherst, Mass.	Amherst Col., June 1936	572	1	
Woodbury, N. J.	New York Univ., Nov. 1936	1159	1	
Tuscaloosa, Ala.	B. Kaufmann, Aug. 1936	545	1	
Lausanne	V. Jollos, July 1936	955	2	0.21±0.09
Seto, Japan	Zool. Inst. Kyoto, Aug. 1936	1236	—	—
Kyoto, Japan	Tokyo Imp. Univ. Kyoto, Sept. 1936	875	1	
		13602	14	0.10±0.02

Analysis of the Florida stock

In order to determine whether the basis for the high mutability rate in Florida stock is genic, the males from the backcross, in which the female parent was ClB/*g pl* and the male parent +Florida, were tested for mutability. Among 1338 X chromosomes tested, four (0.30±0.10 percent) carried a lethal. This test revealed a mutability rate lower than that of the original Florida stock but higher than the average mutability rate of wild stocks. Since all of the males tested had the Florida X chromosome, this experiment showed that if the factor responsible for high mutability is genic, it is not located in the X chromosome.

The next step in these tests was to substitute autosomes of a low mutable stock for the Florida autosomes and thus to determine whether any of the Florida autosomes carry a factor responsible for high mutability. Swedish-b stock was selected for this purpose and stocks with different combinations of Florida and Swedish-b chromosomes were built up. Mutability tests for these stocks are summarized in table 2. It is evident from this summary that only combinations homozygous for the second Florida chromosome had the high mutability. This indicates strongly that our Florida stock carried in the second chromosome a recessive factor which is responsible for the high rate of mutability in that stock.

TABLE 2

Mutability in stocks having only certain Florida chromosomes. (F=Florida; S=Swedish-b.)

CONSTITUTION	NUMBER OF			PERCENT OF LETHALS
	MALES USED	CHROMOSOMES TESTED	LETHALS	
1F 2S 3S	10	1162	—	—
1S 2F 3S	21	1707	17	1.00
1S 2S 3F	10	804	—	—
1F 2F 3S	10	1215	11	0.91
1S 2F 3F	8	790	9	1.14
1F 2S 3F	8	800	—	—

Action of the Florida factor

Detailed analysis of the data reveals interesting information regarding the action of the Florida factor. Table 3 gives a summary of the data obtained in tests of stocks containing Florida and Swedish-b chromosomes. This table contains data obtained from the tests of sperm from individual males and also shows the approximate position of the lethals in the chromosome. It is evident that lethals were found in the sperm of certain males only and also that the lethals carried by the sperm taken from any one male tend to be located in the same region of the chromosome. This grouping of lethals indicates that those of the same group are genetically identical. It can be safely assumed that the genetic change producing a lethal effect occurred sometime during the ontogeny of the male and thus was transmitted by a group of sperm which evolved from the affected cell. The supposition that all lethals of a group are identical is also supported by the fact that in male number 5 of the constitution 1F 2F 3S (table 3) all six changes which were classified as lethal by standard tests in vials proved to be semilethals when flies were raised under better conditions for linkage tests. Similarly a group of nine changes obtained from male 8 of 1S 2F 3S constitution proved to be semilethal and in addition every member of that group showed a similar morphological characteristic expressed as a groove on the abdomen.

From the data giving the number of lethals in a group and the number of sperm tested from each male parent, it is possible to estimate the time in ontogeny when the change, which was the progenitor of the group, occurred. This estimate is reached by dividing the number of sperm tested by the number of changes in the group. In table 4 calculations are given for the time of origin of seventeen independent changes. From this table it is evident that changes are not limited to any one stage of the germ cell development. Changes which occurred when the germ cells were in as early

TABLE 3

Lethals appearing in the sperm of individual males.

MALE CONSTITUTION	NUMBER	NUMBER OF		POSITION OF LETHALS			
		SPERM TESTED	LETHALS	*ec*	*ct*	*v*	*g*
1F 2F 3S	1	171	—				
	2	153	1		1		
	3	53	4			1	3
	4	103	—				
	5	189	6				6*
	6	111	—				
	7	99	—				
	8	96	—				
	9	134	—				
	10	108	—				
1S 2F 3F	1	29	—				
	2	98	6	6			
	3	42	—				
	4	152	—				
	5	127	—				
	6	86	—				
	7	101	3	1		2	
	8	255	—				
1S 2F 3S	1	55	—				
	2	40	1				
	3	36	—				
	4	49	2	2			
	5	75	—				
	6	29	5		3	2	
	7	51	—				
	8	75	9				9**
	9	51	—				
	10	62	—				
	11	72	—				

* All semilethal.

** All semilethal and showing similar morphological characteristic (groove on the abdomen).

as the 8 to 10 cell stage were observed. This is an early embryonic stage corresponding to about the ninth nuclear division, when germ cell nuclei have just become separated from the nuclei which are to form the soma. Several changes were also observed which probably occurred in spermatogonia at a stage of development when many spermatocytes had been differentiated.

From the data of table 4 the approximate frequency of changes at different cell generations can be estimated if the data are arranged so as

TABLE 4

Data indicating the stage in the male germ cell development at which changes occurred.

LETHALS FROM THE MALE	NUMBER OF LETHALS IN THE GROUP	NUMBER OF SPERM TESTED	CHANGE OCCURRED WHEN GERM CELLS NUMBERED ABOUT
1F 2F 3S —2	1	153	153 cells
—3	1	53	53 "
—3	3	53	18 "
—5	6	189	33 "
1F 2F 3F —2	6	98	16 "
—7	1	101	101 "
—7	2	101	50 "
1S 2F 3S —2	1	40	40 "
—4	2	49	25 "
—6	3	29	10 "
—6	2	29	15 "
—7	9	75	8 "
291–87— 1	2	297	147 "
— 3	1	95	95 "
291–93—15	2	58	29 "
291–94—10	2	64	32 "
—12	2	61	30 "

to show the frequency of changes per number of cells, divided into classes following a geometric progression. Such a classification is given in table 5.

TABLE 5

Frequency of changes occurring at different cell generations of germ cell development.

GERM CELL NUMBER	8	9–16	17–32	33–64	65–124	125–
Frequency of changes	1	3	5	4	2	2
Corrected frequency	1	1.5	1.25	0.5	0.125	0.0625

To obtain the actual frequency of change, the data for each successive class were divided by 1, 2, 4, 8, 16, and 32 respectively in order to correct for the increase in the chance of a change occurring in each succeeding class due to the increased number of cells. When this is done "corrected frequency" values (table 5) are obtained showing the approximate frequency of changes as observed during the six cell generations of the development of the male germ cells. Considering the small number of observations, the values for the first three cell-generations are similar. Values for the last three generations are lower than the values for the first three. This, however, should be expected since such late changes can be detected only if a large number of sperm from individual males is tested. This has not always been done in our experiment.

This evidence favors the assumption that the Florida mutability factor stimulates gene changes throughout the whole period of germ cell development. It suggests also that these changes occur with approximately equal frequency at different stages of that development.

Visible gene changes

Special experiments have not been performed to determine the action of the mutability factor on the frequency of visible gene changes. However, linkage tests were made with all lethals found in this experiment and in doing that, the material was inbred for two generations after the original cross between ClB/*ec ct v g* and +Florida was made. In this second generation the mutability factor became homozygous in a certain proportion of individuals and, if effective, should have produced a higher frequency of visible mutations among F_3 offspring which were used in linkage counts.

Among 15,000 individuals examined in linkage tests the following visible changes were found:

yellow	24 times	black	2 times
forked	3 times	blistered	1 time
lozenge	2 times	dwarfish	1 time
vermilion	2 times	curled wings	1 time

This indicates that the Florida mutability factor increases the rate of visible mutations as well as the rate of lethal changes.

A large proportion of yellow mutations among visible changes raises the question as to whether the mutability factor has a specific action or whether it is likely to increase the changes in certain genes to a greater extent than in other genes. Results with lethals show that these are scattered throughout the whole length of the chromosome indicating that a large number of genes must be sensitive to the action of the mutability factor. What appears to be a disproportionally high frequency of yellow mutants may be accounted for in several ways. First of all, in the experiments where these mutants were found, echinus, cut, vermilion and garnet characters were involved which, to a large extent, prevented the detection of new mutant forms expressed as rough eyes, cut wings and certain eye colors. Furthermore, it is probable (PATTERSON 1932, DEMEREC 1933) that the majority of changes in any one locus are lethal changes, presumably deficiencies. If deficiencies for a certain locus are not lethal but if they show up as visible characters, then it might be expected that visible changes in these loci will be more frequent than visible changes in loci where deficiencies are lethal. Yellow seems to be such a "deficient non-lethal" locus. These two reasons, however, may not be sufficient to account for the absence of such distinct mutants as white, miniature, and singed. Therefore, the possibility is not excluded that the mutability factor has a differential effect on various loci.

Of the 24 yellows found, 10 have both yellow setae and yellow body and resemble yellow-1; and 14 have gray setae and yellow body and resemble yellow-2. Both lozenge changes found are sterile in homozygous females. One forked change has slightly forked bristles, and the other has both hairs and bristles strongly forked.

Mutability factor and ontogeny

Evidence was presented earlier indicating that the Florida mutability factor stimulates the occurrence of lethal changes during the whole period of the development of male germ cells. The majority of the visible changes occurred in females, and a large proportion of them showed up with several mutant males. This means that in females, as well as in males, changes occur during the development of the germ cells.

It is known that in Drosophila the fertilized nucleus undergoes about eight simultaneous divisions before a blastoderm is formed. After eight such divisions when there are 256 nuclei in the egg, a few of them, about 5–15, migrate toward the posterior end of the egg to form the rudiments of the germ cells. All changes occurring prior to the separation of the germ cells should, as a rule, produce mosaics affecting both germ cells and soma. Changes occurring after the separation of the germ cells produce somatic mosaics if they occur in somatic cells, and germinal mosaics if they occur in the germ cells.

Among a relatively small number of flies examined, 24 germinal changes to yellow were found. Somatic changes to yellow show up on all setae and if the flies are examined carefully such a change can be detected on a single seta. Since a fly has about 1500 setae, it is evident that it should not require many flies to detect somatic changes if they occur with the same frequency as observed for germinal changes. More than 150 males were carefully examined for yellow setae. Only one yellow-mosaic case was found. Unfortunately, it was lost before it could be rechecked. This indicated that yellow changes in somatic cells occur with much lower frequency than in germinal tissues. This in turn suggests that the action of the Florida mutability factor may be limited to germ cells only. If that is true, then the factor is inactive during the first eight nuclear divisions, its activity being limited to the nuclei developing into germinal tissues.

Normal mutability rate in the Florida stock

About one year after these experiments with the Florida stock were completed another set of experiments was started to test the effect of temperature on the mutability factor, and to accumulate more data on the frequency of changes at different stages of ontogeny. To my great surprise it was found that the Florida line used in these experiments had a very low mutability rate. Seventy-two males were tested individually,

but lethals were found among the sperm of only five of them. In the case of four males, two lethals were obtained from each and in one case one lethal was found. Altogether 6743 sperms were tested and since 5 lethals were found, the rate of change was 0.074 percent.

The original Florida line apparently was heterozygous for the mutability factor and during the year which passed between the two sets of experiments, that factor was almost entirely eliminated from the stock. The Florida stock without that factor, therefore, has a very low rate of spontaneous mutability.

Discussion

It is known that variations in the spontaneous mutability rate do occur. A number of so-called "unstable genes" have been investigated (Demerec 1935), all of which have a relatively high rate of change. If changes responsible for the appearance of mutations are chemical changes, as seems probable, then unstable genes are in such a chemical state that even ordinary environmental conditions are close to the threshold where changes occur. Since only slight variation in the environment is sufficient to bring conditions to this threshold point, it is to be expected that the rate of change in unstable genes would be readily affected by various factors. Such behavior has been described for unstable miniature of *Drosophila virilis* (Demerec 1929a, 1930) where five genetic factors are known to stimulate the rate of mutations in that unstable gene.

A similar interpretation can explain the action of the Florida mutability factor. It can be assumed that this mutability factor influences the gene environment to such an extent that changes in a large number of genes are likely to occur. Variations in the environment as it exists in the different cells are sufficient to cause changes in genes.

Specificity of the gene behavior observed on many other occasions is again evident in the present case. The experimental evidence now available indicates that the changes in genes occur only during the development of the germ cells. This means that in these cells only the mutability factor is able to exert a sufficiently strong influence on the gene environment to bring about a condition which causes a mutation to occur. This situation is similar to that found in the unstable miniature-gamma of *virilis* (Demerec 1929b), this gene being unstable in somatic cells only.

In the material described here, the spontaneous frequency of the X chromosome lethals was 1.09 percent. Since the stock used in experiments was not homozygous for the mutability factor, it is evident that the rate of mutations should be higher in the homozygous material. The observed frequency of lethals, therefore, can be taken as the lowest value for the effect of the Florida mutability factor. This value is equivalent to

the one obtained from the X-ray treatment of sperm by about 300 r-units. Since both the mutability factor and the X-ray treatment produced a similar effect, the question may be raised whether or not both results could be due to the action of a similar mechanism.

It has been noted on several occasions that new spontaneous mutations are discovered in waves, that is, that sometimes a large number of them is found within a relatively short period of time (SPENCER 1935). Presence of a mutability factor in experiments carried on during a mutation wave can readily account for this behavior.

For 29 lethals of independent origin investigated in experiments reported here, genetic tests were made to determine whether they were connected with chromosomal aberrations. In these tests *ec ct v g* stock was used so that reductions in crossing over produced by a chromosomal aberration to the left of *B* should have been detected. Since the region to the right of *B* was not covered in these tests lethals located in that region were not included among 29 lethals considered here. Genetic tests showed that none of the spontaneous lethals was connected with a chromosomal aberration. It is known that lethals induced by X-ray treatment are frequently connected with chromosomal aberrations (inversions and translocations). That does not mean, however, that X-ray lethals should be different from spontaneous lethals. It has been observed that the simultaneous occurrence of a lethal and of a chromosomal aberration increases with the dosage applied and that lethals from low dosage treatments are rarely connected with chromosomal aberrations. This may mean that when the frequency of lethals is low they are independent of chromosomal aberrations and as the frequency increases coincidence of two types of changes increases also (DEMEREC 1937b). According to that interpretation the frequency of spontaneous lethals investigated here has not reached the level at which chromosomal aberrations occur.

The data of table 1 reveal a significant difference in the spontaneous mutability rate between different lines of *D. melanogaster*. Of the fifteen lines tested, three had a significantly higher rate of mutations than the other lines. If the genetic constitution of the line is responsible for the increased mutability, then it appears probable that there are a number of mutability factors acting in a similar manner as the Florida factor. Since such factors increase the variability in the line in which they are present, they may play a significant role in the evolutionary processes of a species.

SUMMARY

Frequency of spontaneous X-chromosome lethals was measured by ClB method in fifteen stocks of *D. melanogaster*. In three of them the frequency was significantly higher than in others, the highest being in the Florida stock.

Genetic tests showed that a recessive second chromosome gene (*mu-F*) was responsible for the high frequency of lethals in the Florida stock. It has been found that this gene also increases the rate of visible mutations.

Data show that this mutability gene acts during the development of germ cells, both in males and females. The available evidence indicates that the rate of action is similar during successive cell generations of the developing male germ cells.

It is suggested that the Florida mutability factor affects the gene environment in such a way as to bring it to the threshold condition at which changes in various genes occur.

Twenty-nine spontaneous lethals of independent origin have been tested by genetic methods for chromosomal aberrations. None has been found.

LITERATURE CITED

DEMEREC, M., 1929a Genetic factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. Proc. Nat. Acad. Sci. **15**: 834–838.

1929b Changes in the rate of mutability of the mutable miniature gene of *Drosophila virilis*. Proc. Nat. Acad. Sci. **15**: 870–876.

1930 A genetic factor affecting germinal mutability of miniature-alpha wing character of *Drosophila virilis*. The Laws of Life, 45–56 pp. Prague.

1933 What is a gene? J. Hered. **24**: 368–378.

1935 Unstable genes. Bot. Rev. **1**: 233–248.

1937a A mutability stimulating factor in the Florida stock of *Drosophila melanogaster*. (Abstract) Genetics **22**: 190.

1937b Relationship between various chromosomal changes in *Drosophila melanogaster*. Cytologia (in press).

PATTERSON, J. T., 1932 Lethal mutations and deficiencies produced in the X chromosome of *Drosophila melanogaster* by X-radiation. Amer. Nat. **66**: 193–206.

PLOUGH, H. H., and HOLTHAUSEN, C. F., 1937 A case of high mutation frequency without environmental change. (Abstract in Genetics **22**: 203) Amer. Nat. **71**: 185–187.

SPENCER, W. P., 1935 The non-random nature of visible mutations in Drosophila. Amer. Nat. **69**: 223–238.

DEVELOPMENT OF EYE COLORS IN DROSOPHILA: PRODUCTION AND RELEASE OF cn^+ SUBSTANCE BY THE EYES OF DIFFERENT EYE COLOR MUTANTS

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INTRODUCTION

THE evidence given in previous papers by the authors has led to the hypothesis that individuals with the mutant eye color vermilion are deficient in two diffusible substances. These have been designated v^+ and cn^+ substances. It has been shown also that either one or both of these substances are necessary for the development of any one of several other mutant eye colors. The method of establishing the latter fact was to transplant eye anlagen from larvae homozygous for vermilion and for the mutant gene in question (*vvxx*) into wild type larvae. Such implants developed the color characteristic of the mutant *x* (that is, $++xx$) and not that of the combination *vvxx*. A genetically vermilion eye-implant was thus modified by the host into a nonvermilion phenotype. The phenotype *x* thus depends upon v^+ or cn^+ substance or both. These are supplied by the wild type host, but not by the genetically vermilion donor. This is not true of the vermilion-like eye color mutants (cardinal, cinnabar and scarlet); here we have no evidence that the double recessives with vermilion are phenotypically different from vermilion.

Eye disks of the mutants bordeaux (*bo*), clot (*cl*), Henna-recessive (Hn^r), mahogany (*mah*), purpleoid (*pd*), prune-2 (pn^2), purple (*pr*), raspberry-2 (ras^2), sepia (*se*), sepiaoid (*sed*), and safranin-2 (sf^2) grown in vermilion hosts give rise to eyes more or less intermediate in color between their own control implants and control implants of the double recessives with vermilion. It was concluded (EPHRUSSI and BEADLE 1937a) that, in these mutants, the eye itself is incapable of producing sufficient v^+ or cn^+ substance, or both, to give the characteristic pigmentation of the mutant.[1] Since we know that a vermilion host is deficient in both v^+ and cn^+ substances, the above experiments do not distinguish between production of the two different substances by the implant. By growing eye-implants in cinnabar hosts, which are deficient in cn^+ substance but not in v^+ substance, it should be possible to make this distinction; this is the primary purpose of the work reported in this paper.

In studies of the release of diffusible substances by eye-implants, it has

[1] Eyes of the mutants brown (*bw*), claret (*ca*), carnation (*car*), cardinal (*cd*), carmine (*cm*), cinnabar (*cn*), garnet-2 (g^2), light (*lt*), maroon (*ma*), peach (p^p), ruby (*rb*), and scarlet (*st*) grown in vermilion hosts develop colors characteristic of their own genotypes.

been shown that of twenty-five eye color types tested, only nine (claret, carmine, cinnabar, garnet-2, light, maroon, peach, ruby, and scarlet) give evidence of release of v^+ substance when tested in apricot vermilion (w^a v) hosts. Release of v^+ substance is indicated by a modification of the eyes of the hosts toward apricot. On the other hand, all of the eight types of eye-implants tested for the release of cn^+ substance (wild type, bright, cardinal, Henna-recessive, sepia, scarlet, vermilion, and apricot) gave positive results (EPHRUSSI and BEADLE 1937c). In this test, cn^+ substance is detected by a modification of the eyes of apricot cinnabar hosts toward apricot. A second purpose of the present paper is to present the results of additional tests for the release of cn^+ substance by eye-implants of various genotypes.

MATERIAL AND TECHNIQUE

The technique of transplantation used in the experiments reported here has been described by EPHRUSSI and BEADLE (1936). The same series of mutants was tested as was previously used in tests for the release of v^+ substance. In all tests eye disks were taken from larvae approaching puparium formation and implanted in apricot cinnabar larvae of approximately the same age. Shortly after eclosion the eyes of the hosts were examined for implant-host effects. Implants were removed from the hosts and compared with controls made at the same time. Thus, in the case of a sepia implant grown in an apricot cinnabar host, after recording the color of the eyes of the host, the implant was compared with a sepia implant grown in a sepia host.

EXPERIMENTAL RESULTS

From the results summarized in table 1 it is seen that in all cases except vermilion and possibly bright, the implant develops pigmentation according to its own genetic constitution; that is, the implant shows autonomous development of color. On the other hand, all implants except cinnabar modify the eye color of the host toward apricot; in such cases the development of color in the host's eyes is not autonomous with respect to the cinnabar component. Implant-host modifications are recorded in a roughly quantitative way. It is seen that this modification varies with the genetic constitution of the implant.

DISCUSSION

It is known from studies of the interaction of different eye color characters that cinnabar in combination with any other eye color except vermilion, cardinal, and scarlet (that is, those belonging to the vermilion group of SCHULTZ, 1932), results in a color different from either component. From these relations we can infer that cn^+ substance is necessary for the

TABLE 1

Development of eye color in implants of various mutants grown in w^a cn hosts, and the effect of the implant on the eye color of the host. Under heading "number of individuals" the arrangement of sex combinations is: ♀ in ♀, ♀ in ♂, ♂ in ♀, ♂ in ♂, and total.

GENOTYPE OF IMPLANT	NUMBER OF IMPLANTS	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	EFFECT ON HOST
+		*	+	strong
bo	1	2, 4, 3, 5; 14	*bo*	medium
bri	1	6, 3, 6, 2; 17	*bri***	very weak
bw	1	6, 4, 3, 8; 21	*bw*	weak
ca	1	6, 3, 6, 6; 21	*ca*	medium
car	1	4, 4, 1, 5; 14	*car*	strong
cd	1	7, 0, 4, 3; 14	*cd*	medium
	2	0, 1, 0, 0; 1	*cd*	medium
cl	1	6, 3, 3, 1; 13	*cl*	weak
cm	1	5, 3, 3, 3; 14	*cm*	medium
cn		*	*cn*	none
g^2	1	4, 4, 6, 4; 18	g^2	strong
Hn^r	1	6, 3, 2, 5; 16	Hn^r	weak
lt	1	3, 5, 5, 3; 16	*lt*	strong
	2	1, 0, 0, 0; 1	*lt*	strong
ma	1	2, 5, 6, 2; 15	*ma*	medium
mah	1	7, 5, 7, 8; 27	*mah*	very weak
p^p	1	2, 1, 3, 0; 6	p^p	strong
pd	1	0, 4, 4, 2; 10	*pd*	strong
pn^2	1	4, 2, 2, 2; 10	pn^2	medium
	2	0, 1, 1, 1; 3	pn^2	medium
pr	1	1, 0, 1, 0; 2	*pr*	strong
ras^2	1	9, 5, 8, 5; 27	ras^2	weak
rb	1	3, 4, 7, 2; 16	*rb*	medium
se	1	5, 4, 3, 4; 16	*se*	strong
	2	0, 0, 1, 0; 1	*se*	strong
sed	1	4, 2, 1, 1; 8	*sed*	medium
sf^2	1	3, 0, 2, 1; 6	sf^2	weak
st	1	2, 2, 4, 1; 9	*st*	strong
?		*	+	strong
w	1	0, 4, 1, 0; 5	*w*	strong
	2	0, 1, 0, 0; 1	*w*	strong

* Data previously published (EPHRUSSI and BEADLE 1937c).

** Implants possibly slightly darker than controls (*bo* implants) grown in *bo* hosts).

development of any eye color not belonging to the vermilion group. On the basis of this argument, it follows that any eye-implant which shows autonomous development of color when grown in a host deficient in cn^+ substance (apricot cinnabar in this case) must itself be able to make the necessary cn^+ substance. Since there is no evidence that cardinal and scarlet eyes need cn^+ substance to develop their characteristic colors, it does not follow from this behavior alone that the eyes of these mutants are

able to produce *cn*$^+$ substance. As pointed out in the introduction, eyes of certain mutants do not show autonomous development when grown in vermilion hosts which are deficient in both *v*$^+$ and *cn*$^+$ substances. It was assumed that such eyes are themselves unable to produce as much of one or the other, or both, of the substances as they would have utilized in their normal environment. The fact that eye-implants of these same mutants grown in apricot cinnabar hosts show autonomous development of color indicates that *v*$^+$ substance and not *cn*$^+$ substance is the limiting factor when they are grown in vermilion hosts. In other words, eyes of all the mutants studied, when grown in a host able to supply them with a sufficient amount of *v*$^+$ substance, produce the *cn*$^+$ substance they would normally use.

The above conclusion is confirmed by the fact that eye-implants of all mutants tested with the exception of cinnabar release detectable quantities of *cn*$^+$ substance. From the experiments on release of *cn*$^+$ substance by eye-implants it is seen that scarlet and cardinal eyes are capable of producing and releasing this substance. Eyes of mutants assumed from other types of evidence to be deficient in *v*$^+$ and *cn*$^+$ substance (carnation, carmine, claret, garnet-2, peach, ruby, and vermilion, BEADLE and EPHRUSSI 1936), release *cn*$^+$ substance when grown in the presence of *v*$^+$ substance. This is in agreement with the previous interpretation of the authors which assumed that in these cases the deficiency of the two substances results fron a partial or complete block in the sequence of reactions leading to the formation of *v*$^+$ substance. Once this substance is supplied, as it is by a cinnabar host, there should be no further block to the formation of *cn*$^+$ substance (EPHRUSSI and BEADLE 1937b, BEADLE and EPHRUSSI 1937a).

The two mutants bright and mahogany show evidence of having reduced amounts of *cn*$^+$ substance, but, according to the corresponding type of test, they show no evidence of having a reduced amount of *v*$^+$ substance (BEADLE and EPHRUSSI 1937b). It has been assumed that in these mutants the reactions leading to the formation of *cn*$^+$ substance are partially blocked. The tests for release of *cn*$^+$ substance by eyes of these mutants were positive but very weak. The argument that *cn*$^+$ substance is produced at a reduced rate in these mutants as compared with wild type is in general supported.

With regard to the observed differences in magnitude of implant-host effects, several interpretations appear plausible. It seems reasonable to assume that at least two factors influence this effect: 1) the total amount of *cn*$^+$ substance produced by the implant, and 2) the amount used by the implant itself. One might assume that the difference between these two quantities represents the amount released. Following these assumptions,

the observed implant-host effects measure only this difference. Since at present we have no way of measuring either the total amount produced or the amount used by the eye itself, it is perhaps best to defer further discussion of quantitative aspects of the problem.

The authors are indebted to M. S. CHEVAIS and to Mrs. JUANITA BALLINGER for technical assistance in carrying out the experiments reported above.

SUMMARY

Larval eye disks of wild type and of the mutants *bo*, *bri*, *bw*, *ca*, *car*, *cd*, *cl*, *cm*, *cn*, g^2, Hn^r, *lt*, *ma*, *mah*, p^p, *pd*, pn^2, *pr*, ras^2, *rb*, *se*, *sed*, sf^2, *st*, *v*, and *w* were transplanted to w^a *cn* larvae. In every case except *v* the implants developed into eyes of a color corresponding to their genotype. Since there is evidence that these mutants, with the exception of *cd*, *cn*, *st*, and *v*, require cn^+ substance for the development of their characteristic colors, it is argued that eye-implants of the mutants tested, when grown in hosts capable of supplying v^+ substance, are themselves able to make the cn^+ substance necessary for the autonomous development of eye color.

All implants tested with the exception of *cn* produced a modification of the eyes of the host in the direction of w^a cn^+. The results of these tests are a direct confirmation of the conclusion stated above.

A comparison is made between these results and the results previously published of the transplantation of eyes of the same mutants to *v* and to w^a *v* hosts. From this comparison it is argued that v^+ substance, not cn^+ substance, is the limiting factor in pigment development in eyes of certain mutants grown in hosts deficient in both v^+ and cn^+ substance, that is, in *v* hosts.

LITERATURE CITED

BEADLE, G. W., and EPHRUSSI, B., 1936 The differentiation of eye pigments in Drosophila as studied by transplantation. Genetics **21**: 225–247.

1937a Development of eye colors in Drosophila: diffusible substances and their interrelations. Genetics **22**: 76–86.

1937b Development of eye colors in Drosophila: the mutants bright and mahogany. Amer. Nat. **71**: 91–95.

EPHRUSSI, B., and BEADLE, G. W., 1936 A technique of transplantation for Drosophila. Amer. Nat. **70**: 218–225.

1937a Development of eye colors in Drosophila: transplantation experiments on the interaction of vermilion with other eye colors. Genetics **22**: 65–75.

1937b Développement des couleurs des yeux chez la Drosophile: Revue des expériences de transplantation. Bull. Biol. Fr. Belg. **71**: 54–74.

1937c Développement des couleurs des yeux chez la Drosophile: influence des implants sur la couleur des yeux de l'hôte. Bull. Biol. Fr. Belg. **71**: 75–90.

SCHULTZ, J., 1932 Aspects of the relation between genes and development in Drosophila. Amer. Nat. **69**: 30–54.

SOMATIC SEGREGATION AND ITS RELATION TO ATYPICAL GROWTH*

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HORTICULTURAL literature records many instances of changes in flowers and fruits during development that are transmitted by vegetative and seed propagation. In plants that are asexually reproduced these variations, however rare, are easily seen and when they have value are perpetuated. Similar deviations from normal occur in plants multiplied by seeds but these are usually not so noticeable because of the greater variability of such plants.

Color and texture alterations are found in the seeds of maize and have been studied for some time on account of their genetic and cytological interest. The most commonly observed changes during the development of the seed are the loss or acquisition of color and alterations in starch composition from the normal well-filled, more or less translucent kernel to the wrinkled condition of the sugary seed or the opaque, red-staining endosperm known as waxy.

PREVIOUS INTERPRETATIONS OF MOSAIC FORMATION

The discovery by GUIGNARD and by NAWASCHIN in 1899 that the endosperm results from a fertilization process, similar to that of the embryo, gave an explanation of the phenomenon of xenia in maize. The supposed influence of the pollen on maternal tissue turned out to be merely an effect upon the filial generation corresponding to dominance of genetic characters in the hybrid progeny. WEBBER (1900) and CORRENS (1901) observed cases where the immediate effect of the pollen was visible in one half of the seed but not in the other. EAST and HAYES (1911) also observed many instances of this kind and called attention to their similiarity to insect gynandromorphs.

Incomplete fertilization

It was suggested at first that these half and half seeds could be due to a failure of normal fertilization such that the endosperm nucleus and the second male nucleus develop separately, or that the male nucleus might unite with one endosperm nucleus and the other develop independently. These suggestions followed a similar interpretation of aberrant development in animals. Both of these assumptions were disproved by EMERSON (1915) who obtained seeds showing characters derived from both parents

* A part of the cost of the accompanying figures is met by the Galton and Mendel Memorial Fund.

throughout the endosperm and loss of other characters from some of the cells. A similar hypothesis of incomplete fertilization put forward by CASTLE (1935) to account for the mosaic fowl described by ROBERTS and QUISENBERRY (1935a) was likewise disproved by the same writers (1935b) on genetic evidence.

Mutation

Although EMERSON recognized the possibility that mosaic seeds might be produced by a process of "vegetative segregation," as previously suggested by EAST and HAYES, he said: " if such a segregation occurs, it is not a typical Mendelian segregation, because, in neither of the three cases reported, could all the independently inherited genetic factors present in a heterozygous condition have been involved. It is suggested that such seeds may also be interpreted as somatic mutations, that is, that they may be due to a change in genetic constitution rather than to a segregation of genetic factors." EMERSON cited numerous cases of bud sports in heterozygous plants where a change in a single gene from the dominant tc the recessive condition could lead to visible alteration.

Somatic mutation is still generally believed by many horticulturists to be responsible for bud variations. J. L. COLLINS (1936) describes frequent changes from a dominant spineless condition to a recessive spiny condition in the pineapple. Cytological examination failed to show any change in chromosome content in the different tissues. Reverse changes from recessive to dominant color appear in homozygous seeds of maize. For the present these can only be interpreted as a germinal change of some kind not clearly related to any process of somatic segregation. Nevertheless, the concept of somatic mutation is inadequate to account for many of the observed facts as EMERSON (1921) clearly showed in a later study of the problem.

In 1913 G. N. COLLINS reported an interesting observation of O. F. COOK's in which a plant of Chinese waxy maize having colored aleurone and waxy endosperm was pollinated by a white seeded variety having normal horny endosperm. The resulting seeds were for the most part colorless and horny. The pollen parent carried the dominant color inhibitor and the normal starch condition. One seed had a colored area in the aleurone and this irregular spot was underlaid by a waxy area of the same size and corresponding outline. COLLINS observed another mosaic-pattern seed with a similar coherence of characters. In this case the pollinator had colored aleurone and sugary endosperm. The seed parent was waxy and colorless, lacking the inhibitor. The resulting mosaic seed was non-sugary throughout showing that all the endosperm cells had developed from a fusion. A colorless aleurone spot was underlaid by an opaque waxy area. Thus

somatic cells showed linkage of characters according to the way these characters came into the hybrid from the parents.

Non-disjunction

At that time the genes involved and their linkage relations were not known. Later, EMERSON (1921) reviewed the evidence to show that these genes are in the same chromosome and pointed out that the results obtained would be expected if the chromosome carrying both dominant genes was removed from one cell during development on the assumption that this deficient cell was able to go on dividing at an approximately normal rate. Pursuing this line of investigation EMERSON presented convincing evidence to show that chromosome behavior was responsible for many cases of mosaicism in maize and in other organisms as well. In the meantime, BRIDGES (1914) had shown that non-disjunction of chromosomes occurred in Drosophila and that this was the explanation for many of the insect gynandromorphs.

In 58 mosaic seeds involving both *C* and *Wx*, EMERSON found 55 cases where these two linked genes were both removed from corresponding areas of aleurone and endosperm making the altered tissue colorless and waxy. In three cases there was a loss of color from the aleurone cells but no change in endosperm texture. The linked genes *C* and *Sh* (shrunken endosperm) were also found to be removed together.

EMERSON was careful to interpret his results in terms of aberrant chromosome behavior without committing himself to any specific hypothesis. He says: "while the writer feels that the genetic evidence in favor of the hypothesis of non-disjunction, or at least of some aberrant chromosome behavior giving a similar result, as the cause of most cases of the kind of aberrant endosperm here discussed is as convincing as such evidence can well be, it is realized that direct proof must come, if at all, from cytological studies."

Deletion

After noting additional exceptional instances where genes on the same chromosome were not removed together (JONES 1935) it seemed probable that non-disjunction was not the explanation for many mosaics. The evidence also indicated that genes near the ends of the chromosomes were lost more frequently than those near the spindle fibre attachment point. From this it seemed more likely that mosaics result from the removal of part of a chromosome rather than the loss of a whole chromosome. MCCLINTOCK (1932) has given cytological evidence to prove that chromatin losses result in color mosaics in plant tissue. STADLER (1933) has shown that gene deletions do occur in maize and that cells with parts of chromosomes missing are viable.

PAIRED CHANGES

The recent discovery of paired mosaics in plants (JONES 1936) has brought new light to the problem. It shows that neither mutation, nondisjunction nor deletion is adequate to account for all the genetic changes that occur naturally in a maize seed during its short period of growth. Paired alterations, or twin spots, as they are conveniently called, have been found to be associated with shifts of the *C*, *C*^{I}, *Pr*, *P*, *Wx* and *Su* genes.

Twin spots involving the C gene

The *C* gene, located on chromosome 9, is one of several complementary color factors in maize. When the other complementary genes are homozygous, one *C* allele in most stocks produces a light color in the aleurone layer. The shade and depth of color varies greatly in different plants in the

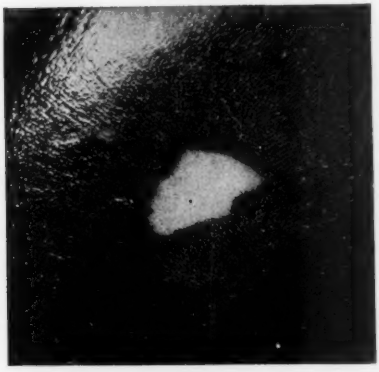


FIGURE 1.—A colorless spot in the aleurone layer of a maize seed resulting from the removal of the *C* gene from one cell and its subsequent growth. ×10.

FIGURE 2.—Paired colorless and dark areas resulting from a shift of the *C* aleurone color gene. ×10.

mature seeds. With recessive *pr* the color is usually a light red. With dominant *Pr* the color varies from a reddish black to a bluish black. When the *C* gene comes from the male parent and is present singly the color is nearly always lighter than in the reciprocal cross from the same plants where two *C* alleles are contributed by the female parent. Homozygous seeds with three *C* alleles are still darker.

Aleurone twin spots were first noticed when seeds of *ccC* composition were examined under a low power microscope. They also occur in large

enough areas to be visible without magnification but had entirely escaped observation. An unpaired colorless spot and a paired colorless and adjoining dark area are shown in figures 1 and 2. Camera lucida drawings

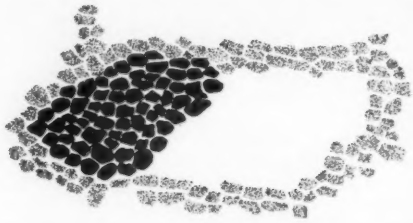


FIGURE 3.—Camera lucida drawing of a colorless area paired with a dark area. A border area of normal cells is represented by the dotted cells. ×80.

FIGURE 4.—Small paired mosaics with the colorless cells drawn in outline. ×160.

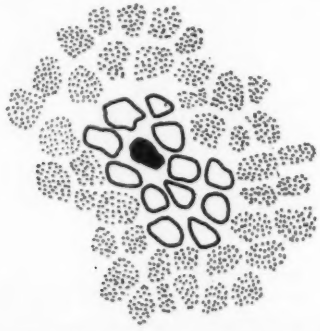


FIGURE 5.—Paired mosaic with one dark cell. ×160.

of other paired, uncolored and dark colored, areas are shown in figures 3, 4 and 5. The dark and colorless patches are usually similar in size and outline. In many cases of irregular pattern one part is a mirror image of the other. Obviously both tissues have a common origin, presumably from a

single cell that in an earlier stage has undergone an unequal mitosis such that one *C* allele was removed from one daughter cell and doubled in the other.

In size these paired mosaics range from nearly the entire surface of the seed down to a pair of single cells. They are usually smaller than the unpaired colorless spots on the same seeds and are seldom large enough to be seen without a microscope. Although the two paired sections are usually equal in area this is not always true. The inequality varies on either side down to single cells as shown in figure 5. Apparently, a mosaic that starts as a twin spot may fail to have any of the *CC* cells surviving to the surface where the color can show and the result is an unpaired white spot. If this were the case unpaired dark spots would also be expected. When looked

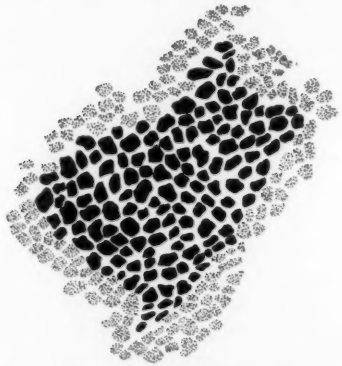


FIGURE 6.—Unpaired dark spot. ×80.

FIGURE 7.—Unpaired colorless spot. ×80.

for they are easily seen as reproduced in figure 6. This may be compared with the unpaired colorless spots which may occur on the same seeds as shown in figure 7. In some cases the two daughter cells of an unequal mitosis have separated and developed in different parts of the seed.

The frequency of these three types of mosaics involving *C* are given in tables 1, 2 and 3. Each line in the tabulation represents an ear resulting from the pollination of *c* by *C* plants. Ears having the same parentage are grouped in one table and totaled below. Each seed is counted once for every type of mosaic it shows regardless of whether there is more than one mosaic of a kind on a seed. In the material first examined there was rarely more than one mosaic of a kind on a seed. Later other material was found that showed many changes on a single seed. The frequency with which the various mosaics appear has little comparative value. Much depends upon the condition of the seed, the nature of the pigment and the transparency of the overlying pericarp. In some progenies mosaics are easily seen in

TABLE 1

Frequency of mosaic areas in colored aleurone seeds having the composition c c C pr pr Pr.

NUMBER OF SEEDS	UNPAIRED			PAIRED						LARGE CELLS	DEPRESSED AREAS	RAISED AREAS
	WHITE	RED	DARK	DARK WHITE	DARK RED	DARK LIGHT	RED WHITE	WHITE LARGE	RED LARGE			
480	186	78	11	26	3		7	4	2	68	11	2
355	131	52	2	11	2		1	2	1	19	14	2
180	32	12	2	6						17	4	1
283	54	13		5		1		1		33	4	3
254	61	20	1	10						22	2	3
193	67	9	1	6						18	3	1
287	51	2		6				2		4	4	1
433	77	16		13		5				31	13	3
2465	659	202	17	83	5	6	8	9	3	212	55	16

TABLE 2

Frequency of mosaic areas in colored aleurone seeds having the composition c c C Su Su su.

NUMBER OF SEEDS	UNPAIRED		PAIRED DARK AND WHITE AREAS	LARGE CELLED AREAS	DEPRESSED AREAS	RAISED AREAS
	WHITE	DARK				
100	30	2	15	1	4	
37	16		3			
50	19		7		1	1
70	27		5			
123	66	4	7	5	1	1
196	58	3	4	1		1
72	29	6	10	2	1	
12	2					
24	2	1	2			
196	81	7	17	8	2	1
38	18		4			
918	348	23	74	17	9	4

TABLE 3

Frequency of mosaic areas in colored aleurone seeds having the composition c c C sh sh Sh.

NUMBER OF SEEDS	UNPAIRED		PAIRED DARK WHITE	LARGE CELLS	DEPRESSED AREAS	RAISED AREAS	WHITE SHRUNKEN AREAS
	WHITE	DARK					
340	79	1	13	3	2	1	1
353	57	3	8	1			
333	4		2				
200	24		6		1		
169	1						
1395	165	4	29	4	3	1	1

areas as small as single cells. In others, many-celled mosaics may be overlooked. The change from colorless to color is much more easily detected than the reverse change and the latter in turn is more noticeable than any variation from dark to light, black to red, or the reverse alterations.

In tables 1 to 3 the seeds are also heterozygous for other genes but these need not be considered now. The aleurone color variations which may be attributed to the aberrant behavior of the *C* gene are as follows:

DATA FROM TABLE	TOTAL NUMBER OF SEEDS	UNPAIRED WHITE	PAIRED WHITE AND DARK	UNPAIRED DARK
1	2465	659	83	17
2	918	348	74	23
3	1395	165	29	4
Total	4778	1172	186	44
4	2284	403	52	52

About 1 in every 4 seeds on the average has an unpaired colorless area; about 1 in 26 a twin spot; and 1 in 109 a dark spot. The dark part of a twin spot is usually less well differentiated from the adjacent normally colored cells than the colorless part. For that reason it would be expected that many of the unpaired dark spots would escape detection. This is brought out in table 4. In these cross-pollinations the F_1 seeds are heterozygous for three basic color genes, each dominant gene coming from the male parent and represented only once. The resulting seeds are so light in color that some seeds could hardly be distinguished from colorless seeds. In spite of the fact that there is three times the opportunity for colorless mosaics to appear, since the loss of either *A*, *C* or *R* results in colorless cells, fewer unpaired and paired mosaics were found but the proportion of dark celled areas was increased from 1 in 109 to 1 in 44 seeds. Nevertheless, the number of dark areas is still far from equalling the number of colorless areas. Also in size the dark areas are smaller than the colorless areas on the average and correspond in their range in size to the dark part of twin spots. From this it seems probable that only a small proportion of the unpaired colorless areas start as paired mosaics of which the dark part fails to reach the surface. If this is true the unpaired colorless areas have different origins in which mutation, non-disjunction, deletion, translocation, somatic crossing over and probably other unknown deviations from normal development all play a part.

Twin spots involving the C^I gene

The most clearly delineated twin spots are exhibited in seeds heterozygous for the aleurone color inhibitor. This gene shows the same linkage with other genes on the same chromosome as *C* (EMERSON, BEADLE, and

FRASER 1935) and the two may be considered to be allelic. The evidence from these mosaic studies gives no indication to the contrary. One C^I allele usually prevents all noticeable aleurone color when all other essential genes are present. When examined under the microscope nearly all such seeds show some pigment in the form of lightly colored granules. Different families vary considerably in the amount of color that develops. The same closely inbred inhibitor stock was used as the male parent in all crosses. In the lightly colored seeds paired colorless and dark areas stand out clearly. The dark spots are evidently cells from which the C^I gene has been removed. The adjacent and corresponding colorless areas evidently have two C^I alleles and their combined action is able to prevent all color. Here also unpaired colorless and dark areas are visible. The dark areas are more numerous than the colorless. Dark spots are easily seen even when they include only a single cell. In some progenies one or more mosaic areas of this kind can be seen on practically every seed and some seeds have fifty or more colored spots varying in size from single cells to half the surface of the seed and rarely nearly the entire surface.

Twin spots involving the Pr gene

The *Pr* gene changes the aleurone color from red to purple when the seed first matures. As the seed dries out the color changes to a dark bluish red or bluish black. There is much variation in color from red to black but no matter what the color is the loss of *Pr* is usually quite apparent. In *Pr* seeds that are reddish *pr* spots are light red. The frequency of mosaics in seeds heterozygous for *Pr* with the other aleurone factors for color present, either in the homozygous or heterozygous condition, is given in tables 1, 4 and 5. In a total of 6070 seeds there are 358 with one or more clear red spots. In 12 other instances well defined dark areas were seen adjacent to the red areas. Doubling of the *Pr* gene evidently makes cells darker in the same way that the *C* gene does but the effect is not so clear-cut and many twin spots no doubt escape detection.

Twin spots involving color modifying genes

In tables 1, 4, 5, 6 and 7 paired dark and light spots are recorded. Unpaired light spots are also given in tables 5 and 6. It is possible that some of these have been confused with red spots but many occur on seeds that are homozygous for *pr* or *Pr*. There are several genes that are known to vary the intensity of aleurone color and probably more exist that have not been located. Anything that alters the growth rate or general activity of the cell may bring about a change in the intensity of color. The fact that these color modifiers are shifted in the same way as the better known aleurone genes is well established.

TABLE 4

Frequency of mosaic areas in colored aleurone seeds having the composition a a A c c C r r R pr pr Pr.

NUMBER OF SEEDS	UNPAIRED			PAIRED				LARGE CELLS	DEPRESSED AREAS	RAISED AREAS
	WHITE	RED	DARK	DARK WHITE	DARK RED	DARK LIGHT	RED WHITE			
388	147	38	13	15	3	3	1	1	9	
162	52	3	1	2			1	2	1	2
130	16		4	2					1	
173	28		5	6						
269	19		13	3					1	
320	59	1	2	8						1
164	21	1	4	4	1	1			1	1
217	14		2	2					16	1
164	21		2	5						
297	26		6	5					3	2
2284	403	43	52	52	4	4	2	3	32	7

TABLE 5

Frequency of mosaic areas in colored aleurone seeds having the compositon pr pr Pr.

NUMBER OF SEEDS	UNPAIRED			PAIRED			LARGE CELLS	DEPRESSED AREAS	RAISED AREAS
	WHITE	RED	LIGHT	DARK WHITE	DARK LIGHT	DARK RED			
216	2	29	2	1		3	2		8
206	2	29	4		2		1	2	
329	2	38	2		2			1	
220		14	1					1	3
177	1	1					1	2	3
69		1	1						1
81		1							
23									
1321	7	113	10	1	4	3	4	6	15

TABLE 6

Frequency of mosaic areas in colored aleurone seeds having the composition C C c sh sh Sh.

NUMBER OF SEEDS	UNPAIRED			PAIRED		LARGE CELLS	DEPRESSED AREAS	RAISED AREAS	SHRUNKEN AREAS
	WHITE	LIGHT	DARK	LIGHT DARK	LIGHT LARGE				
249	4	42	1	17	2	4	4	2	
213	2	7	1	5			5	1	1
166		4		1		3		2	
146	1	5				2	3	1	
127		5	2	1		1	8	1	
214	4	28	2	6			4	5	
174	8	2	1	3			3	1	
89	3	3		1		10			
75	1	3							
86									
204	1			1				1	
1743	24	99	7	36	2	20	27	14	1

TABLE 7

Frequency of mosaic areas in colored aleurone seeds having the composition C C c su su Su.

NUMBER OF SEEDS	UNPAIRED			PAIRED LIGHT DARK	LARGE CELLS	DEPRESSED AREAS	RAISED AREAS	SUGARY AREAS
	WHITE	LIGHT	DARK					
199	3	3		1	2	1	2	3
212	1	11		2	2		2	6
105			1			5		
151	2	4		1	2		1	2
240								9
132	2	3				1	2	1
89	2	6		2		1	1	2
73								1
1201	10	27	1	6	6	8	8	24

Aberrant behavior of the Su gene

Mosaics involving the sugary gene are easily seen when they occupy a large enough area but are difficult to detect when small without special treatment. In table 7 the frequency of well marked sugary mosaics is given as 24 in 1201 seeds. When these mosaic areas are cut with a file and stained with a fresh, weak solution of iodine and potassium iodide the mosaic sector is sharply delineated. In contrast to the surrounding starchy tissue the sugary cells stain more slowly and in some material very lightly. The final color is brownish in strong contrast with the bluish black stain of normal starch. Sugary cells are elongated and compressed when dry and swell quickly after absorbing water from the stain. In homozygous waxy seeds or in waxy mosaics, sugary cells are colorless, or very nearly so, after staining lightly. Single cells stand out clearly in strong contrast to the reddish stain of waxy endosperm when treated with iodine.

In some seeds sugary twin spots can be made out indistinctly. Adjacent to sugary areas there are areas of approximately the same size and outline that stain more darkly than normal and presumably these are due to a doubling of the *Su* gene. In homozygous waxy tissue these sugary twin spots are more noticeable.

Aberrant behavior of the Wx gene

In the same way the waxy gene also produces twin spots. Waxy mosaics are very clear. In many seeds they can be seen through the transparent pericarp. When cut with a file they stand out as a white opaque area contrasting with the translucent corneous endosperm having the normal *Wx* allele. In varieties having soft opaque starch, waxy mosaic areas are not easily apparent until stained with iodine. After staining they stand out clearly as colorless or reddish spots and may be differentiated in single cells. In a few cases adjacent areas stain more darkly than normal tissue.

Sometimes the dark area is visible for only a short time after staining. Like the sugary twin spots these waxy and dark paired mosaics seem to be due to a doubling of the normal dominant allele. In homozygous sugary tissue the waxy twin spots are more noticeable.

Aberrant behavior of the Sh gene

Seeds homozygous for *sh* are filled with large opaque endosperm cells in the center of the seed which shrink on drying until the seeds are either hollow in the center or the walls come together leaving the seed much shrunken. The outer part of *sh* endosperm stains no differently from normal cells but the opaque starch usually stains a dark rose red, distinct from waxy. Shrunken mosaics do not show on the surface unless they occupy about a fourth or more of the seed. When both *C* and *Sh* are lost together the white spots may have hollow or depressed areas underneath them but there is no change in the starch reaction in the tissue immediately adjacent to the colorless area. In this respect shrunken mosaics differ distinctly from waxy and sugary mosaics. No evidence of doubling of the shrunken gene has been noticed and from the nature of the change it is doubtful that twin spots could be detected if they did occur.

The change from yellow to white endosperm

The *Y* color gene is lost much less frequently. In 3676 seeds examined (JONES 1935) only one colorless area was found. These seeds were not examined with a microscope and no doubt more small spots might be found. The yellow color is not so sharply restricted to individual cells. It is difficult to make out the border between yellow and white cells. No twin spots involving the *Y* gene have been found.

Twin stripes involving a pericarp color gene

Paired changes in pericarp color have been recorded by EYSTER (1924, 1925). In orange colored seeds varying from light to dark he finds equal and adjacent stripes of pericarp that are light or colorless at one side and darker on the other than the remaining part of the seed. EYSTER interprets these as changes in the *P* pericarp gene itself. He states that homozygous dominant seeds are not noticeably darker than heterozygous seeds.

The writer has found similar paired stripes in seeds of a variety of dent maize known locally as Sweepstakes that varies widely in pericarp color from very light red to almost black. Here also the paired stripes are lighter in one and darker in the other than the remainder of the seed. In some of these cases it may be a color modifying gene that is shifted and not the *P* gene itself. However the *P* pericarp color factor is known to have many alleles varying greatly in depth and arrangement of color and any plant may be heterozygous in respect to these.

Similar paired mosaics occur in fruit epidermis. In figure 8 paired dark and light stripes are shown in apples. In one case the two altered tissues have grown at about the same rate. In the other specimen the dark area is much wider and seems to be grown out whereas the light sector is narrow and somewhat depressed. In both cases small areas within the dark sector have lost color. The light part seems to be stable for its color while the dark part is not. DAHLGREN (1927) also illustrates twin stripes in apples. In one mosaic fruit exactly one quarter is darker and one quarter lighter than the remaining half. Apparently a gene shift occurred at the second division of the fruit bud primordial cell. Other fruits are shown of which one half is light and one half dark, the dividing line passing through the middle of the stem and blossom end.

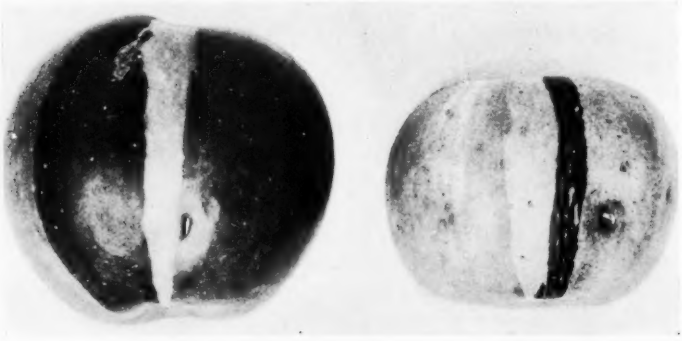


FIGURE 8.—Paired light and dark stripes in apples associated with unequal growth. (Photograph by Dr. GEORGE L. ZUNDEL.)

Vegetatively propagated fruits are notoriously heterozygous. Seedling apples rarely have the same color. Paired stripes may be expected in all fruits and flowers that are heterozygous for color in the epidermis. Citrus fruits show many changes in color and texture of the surface and some of these are paired alterations (SHAMEL, SCOTT and POMEROY, 1918; FROST 1926). Since these paired stripes in maize pericarp and fruit epidermis correspond closely in appearance and behavior to the twin spots in aleurone and endosperm where linked genes accompany the shift of the color genes, EYSTER'S interpretation of this phenomenon as due to a sub-division of the gene itself should be re-examined in the light of this new evidence.

Variation in twin spots

The loss of color in the dark part of the twin stripe in apples is significant. Similar variability is seen in the dark part of the *C* aleurone twin spots in maize. In some cases these color changes are secondary twin spot-

ting as shown in figure 9. The dark part may change to colorless, to darker or may return to normal. Adjacent areas of colorless, light and dark tissue are occasionally seen forming triple spots. The light area differs from the surrounding unchanged part of the seed.

Discussion of paired and unpaired changes

No single hypothesis is adequate to account for all the paired and unpaired mosaics that have been found in seeds of maize having, after fertilization, the same genetic composition. In the paired changes there is apparently not a loss of genes but instead a shift of some kind removing one or more dominant genes from one daughter cell and adding them to the other daughter cell, both of which have therefore resulted from an

FIGURE 9.—Secondary twin spotting. Paired colorless and dark areas within the dark part of a primary twin spot. ×10.

unequal mitosis. If both daughter cells are viable their further equal division produces paired areas differing from each other and the surrounding cells in genetic composition. These areas may be adjoined or separated. One or the other daughter cell may fail to survive thereby producing an unpaired area.

This unequal mitosis may or may not involve chromosome aberration. If it is the result of somatic crossing over of homologous chromatids at a multiple strand stage as occurs in Drosophila (STERN 1936) there need be no loss of chromosomes or parts of chromosomes. Heterozygous allelic pairs are changed to homozygous pairs by a normal process of interchange, allowing recessive characters to appear. Where the homozygous dominant condition differs visibly from the heterozygous condition paired changes result. Somatic crossing over is not the only way in which twin spots or twin stripes can appear. Non-disjunction and translocation can remove genes from some cells and add them to others. But paired mosaics defi-

nitely rule out mutation and deletion as possible explanations. However, somatic crossing over offers no solution for recessive mosaics returning to the dominant condition. In a few cases this apparently does occur. Somatic crossing over also does not account for recessive characters showing in homozygous dominant tissue and these do appear as listed in table 5.

In table 1 the 878 seeds with one or more unpaired spots found among 2465 can be due to mutation, deletion or some form of chromosomal shift such as non-disjunction, translocation or somatic crossing over, provided the cells receiving the additional dominant genes fail to live or do not reach the surface. This is not likely to be the case and chromosomal shift is probably not the explanation for many of these unpaired spots. The 102 paired color changes, many of which occur on the same seeds as the unpaired spots, can not be due to mutation or deletion and are the result of a shift of genes in some manner, the exact nature of which can not be determined in this material. The color changes paired with a doubling in cell size seem to be a shift of chromatin, the nature of which is not known. The large cells unaccompanied by adjoining color changes could be due to a doubling of all chromosomes, but in size of cells and in area of altered tissue they do not differ from the other large cells that are paired with color changes.

There is always the possibility that paired changes are coincidences. Both color changes and cell size changes are frequent and could occur, although rarely, in adjoining cells at about the same time. But the equality in size and the mirror-imaging of many paired mosaics (see figure 2) is evidence that the two parts originated from a single cell.

Depressions are areas of tissue that fail to store starch as shown by the iodine test. They result from the growth of defective cells which shrink at maturity more than normal cells. These defective cells sometimes break down and disintegrate during the development of the seed as shown by the remains of cell walls lining empty cavities underneath the transparent pericarp. They are not the result of a cell lethal since any cell that fails to live would be replaced by neighboring cells and presumably would leave no trace in the mature seed.

The raised areas contain cells that stain darker than the surrounding tissue and indicate the presence of an increased amount of starch. A raised area is sometimes paired with a depressed area and apparently a shift of cell-regulating material has taken place.

In tables 2 and 3 the changes are the same as in table 1 for the *C* gene. *Pr* is not involved. Depressed areas are no more frequent in table 2 than in table 1 and are not due to the uncovering of the sugary gene, the dominant allele being present in duplicate. Table 4 shows the same mosaics as in table 1. The frequency is lower because the extremely light color makes it difficult to detect color variations.

In spite of the fact that all basic color genes are present in the homozygous dominant condition table 5 shows 7 colorless areas in 1321 seeds. Similar mosaics without color have been found in other homozygous material not tabulated. In some cases this colorless surface tissue is slightly depressed indicating a possible failure of the aleurone cells to develop. In other cases there is no irregularity. The aleurone cells are present but without color.

This may be a case of mutation to the dominant color inhibitor but in 2237 seeds examined (JONES 1936a) for mutations of the dominant inhibitor C^I to its color producing allele C no changes were found, making this possibility improbable. If there is an inequality in chromosome distribution it must affect color genes in three homologous chromosomes, a situation that is difficult to visualize. It seems more probable that some defect has prevented the production of color without affecting the growth of the cells.

In tables 6 and 7 plants with colored seeds were used as the female parent and colorless seeded plants for the male parent. In the resulting aleurone there were two C and one c alleles. Out of a total of 2944 seeds, 34 clear white spots were found, along with 126 light colored areas, 8 dark colored areas and 42 paired light and dark spots. In a few cases the white spots were adjacent to or surrounded by light areas and could be understood as the successive loss of dominant C from two homologous chromosomes but in the other cases this is not true, there being but one change from color to no color.

The exact determination of the cellular alterations responsible for these many different kinds of mosaics will have to wait for further genetic and cytological evidence. Additional genetic evidence will be given in the following sections derived from the behavior of linked genes.

THE BEHAVIOR OF *C* AND *Wx* IN A TRANSLOCATION STOCK

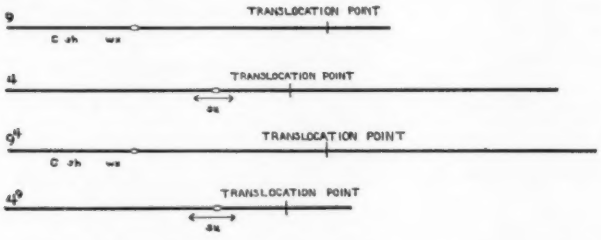
In table 8 the mosaics are recorded that resulted from plants homozygous for *c*, *pr*, *wx* and *su* pollinated by their dominant alleles. The pollen

TABLE 8

Frequency of mosaic areas in colored aleurone seeds having the composition c c C pr pr Pr wx wx Wx su su Su, the pollen parent having the T4-9a translocation.

NUMBER OF SEEDS	UNPAIRED				PAIRED				SUGARY AREAS	DEPRESSED AREAS	RAISED AREAS
	WHITE	RED	DARK	LIGHT	DARK WHITE	DARK RED	DARK LIGHT	RED WHITE			
173	87	33	9	3	229	3	5		1	5	
217	414	101	13	11	1317	8	18	6		10	2
401	403	59	22	18	1121	8	16	4	1	8	
205	314	65	3	13	587	8	7	2		6	
321	508	77	13	7	1361	3	9	6	3	4	
132	38	32		3	2	2	5			2	
109	63	20	1	1	173		3				
1558	1827	387	61	56	4790	32	63	18	5	35	2

parent, furnished by Dr. E. G. ANDERSON, also carried a piece of the number 4 chromosome translocated to number 9 and attached at the opposite end from the *c wx* arm. It was hoped at the time the pollinations were made that the translocation included the *Su* gene. Genetic evidence obtained later by Dr. ANDERSON showed this not to be the case. The *Su* gene was in its usual place but the end of that chromosome from a point beyond the sugary locus was exchanged for a shorter piece of number 9 as shown in the diagram below. The resulting number 9 chromosome is considerably larger and number 4 shorter than normal (This translocation is designated as T 4–9a).



When the crossed seeds were examined it was seen at once that the frequency of many kinds of mosaics was greatly increased over any material previously counted. Nearly all seeds showed one or more changes of some kind. An attempt was made to count all mosaics that could be clearly identified. Since these varied in size from many to single cells it was difficult to make an accurate count. In some places the opaqueness of the pericarp made classification of the type of change doubtful. Attempts to remove the pericarp were not successful in many places. Many indistinct small mosaics involving a few cells were ignored.

The most obvious fact is the relative frequency of the paired dark and white mosaics in relation to the unpaired white spots. In 1558 seeds there were 4790 dark-white twin spots and 1827 white spots. Thus there were nearly three times as many paired mosaics as unpaired. In all the other tabulations paired mosaics are less frequent than unpaired. Another striking result is the low number of sugary mosaics, 5 in 1558 seeds. The data in table 7 from other material show 24 sugary mosaics in 1201 seeds and here only large, clearly identified mosaics were counted. In the translocated stock all depressions were cut and stained with iodine and probably very few sugary mosaics were missed as they were particularly looked for.

The proportion of red (*Pr*) mosaics was not greatly increased but the frequency of dark and light areas was somewhat greater. Comparison is difficult because of the difference in the manner of counting. Seldom were there more than one light or dark spot on a single seed so that a comparison

with the figures in the other tables is justified. It seems certain that the number of mosaics involving the shortened arm of the *Su* chromosome is reduced and the number involving the normal arm of the lengthened *C Wx* chromosome is greatly increased.

LINKAGE OF *C* AND *Wx* IN SOMATIC CHANGES

All of the colorless areas on the seeds listed in table 8 and some additional ears of the same parentage were tested for waxy where the mosaic spot was large enough to be seen easily. The seeds were cut with a file and stained as previously described. In this material the waxy-normal differentiation is quite clear with no possibility of mistaking the two kinds of

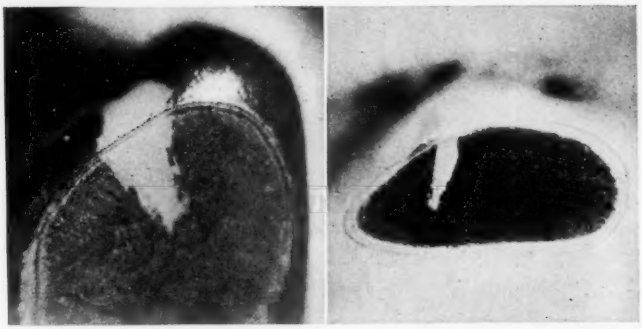


FIGURE 10.—Simultaneous losses of *C* and *Wx* in unpaired (left) and paired (right) mosaics. The colorless areas in the aleurone beneath the transparent pericarp is the result of the loss of *C*. The light area in the subsurface area, stained with iodine, is the result of the loss of *Wx*.

tissue. Photographs of *c* and *wx* mosaics are shown in figure 10. In most cases the margins of the colorless aleurone coincide exactly with the waxy endosperm underneath. In a few cases there is some overlapping. In general these mosaics show clearly that the aleurone is associated by direct cell lineage with the endosperm and this association holds for all parts of the caryopsis.

In the 338 unpaired white spots examined, 55 percent are normal starchy and 45 percent waxy. This is a separation of *C* and *Wx* in a little over half of the mosaics. In 786 twin spots the disassociation of *C* and *Wx* is 49 percent. Germ cell crossing over between these two loci is 26 percent. In somatic segregation, separation of these genes is about twice as frequent as in germinal segregation.

THE EFFECT OF X-RAYS UPON ENDOSPERM MOSAICS

Seeds heterozygous for *c pr wx su* were treated with X-rays shortly after fertilization. The time of treatment and length of exposure varied but

TABLE 9

Linkage of C and Wx in colorless mosaic areas in seeds having the same composition as in table 8.

UNPAIRED WHITE AREAS		PAIRED DARK WHITE AREAS	
Wx	*wx*	*Wx*	*wx*
21	5	26	32
16	14	22	43
26	20	29	34
10	17	33	32
3	5	11	12
39	27	95	92
41	25	113	108
8	8	9	8
15	22	35	36
2	5		
5	4	9	7
186	152	382	404
55.0%	45.0%	48.6%	51.4%

made no significant difference in the frequency of mosaics. The seed stocks were furnished, grown, pollinated and the treatments made by Dr. L. J. STADLER at Columbia, Missouri. Because of unfavorable weather the ears were poorly developed, the seeds small and lightly colored and the aleurone was somewhat obscured by opaque pericarp. In spite of these handicaps the frequency of mosaic areas was seen to be greatly increased. No attempt was made to count all of the changes that were found. As compared to untreated ears of the same constitution the mosaics covered larger areas, they involved many more genes in adjacent tissues and were accompanied

TABLE 10

Linkage of C and Wx in colorless mosaic areas in colored aleurone seeds radiated after fertilization.

X-RAY DOSAGE r	NUMBER OF SEEDS	UNPAIRED WHITE AREAS		PAIRED DARK WHITE AREAS		PAIRED RED WHITE AREAS	
		Wx	*wx*	*Wx*	*wx*	*Wx*	*wx*
300	64	9	15	5	7	4	10
300	90	5	20		3	2	5
300+600	103	6	22		6	1	
300+600	100	5	40	3	12	5	7
600+600	93	2	13	1	8		1
600+600	110	11	40	5	13	4	7
Total	560	38	150	14	49	16	30
			79.8%		77.8%		65.2%

by extensive alterations in growth rate and cell arrangement. Complex paired mosaics resulting from gene losses in several chromosomes were much more frequent than in any other material examined. These will be described later. The *C Wx* association in the colorless part of unpaired and paired areas is given in table 10. Separation occurred in 20 percent of the unpaired white spots, in 22 percent of the paired white and dark spots and in 35 percent of the paired white and red areas. The linkage of these two genes is much closer than in the untreated material and about the same as in germinal crossing over.

If the unpaired dark spots, described previously, start as twin spots of which one part fails to reach the surface, then this submerged part should sometimes show as waxy in seeds where *c* and *wx* are together. Recessive *c* can not show until it reaches the aleurone at the surface. Waxy does not show in the aleurone but does show in any of the cells beneath. When dark aleurone spots are followed down toward the center of the seed by removing successive layers of endosperm and staining each time, waxy cells are found. In a few cases the endosperm below the dark aleurone stains slightly darker than the surrounding tissue. Where this dark tissue leaves off the waxy cells begin. Out of 15 such dark spots examined in this way 6 were found to have waxy cells below in such a position as to indicate that the dark staining and red staining cells had a single cell origin. The submerged waxy cells are few in number and apparently have not divided at the same rate as the other cells.

Similarly, dark staining *Wx Wx* tissue should be located below unpaired white, waxy spots. A few such dark spots have been seen in such a position as to indicate a relationship of this kind. A double dose of the normal allele of waxy is so little different from a single dose that it is difficult to see any difference in most seeds.

SECONDARY TWIN SPOTS

In heterozygous *c c C wx wx Wx* seeds the white and dark paired spots that show secondary twinning in the dark area were examined for the behavior of the linked *Wx* gene. It was found that both *C* and *Wx* may be removed the first time and *C* alone the second as diagrammed in figure 11a; or *C* may go alone the first time and both the second (b); or *C* may go alone both times (c); or *C* and *Wx* may go together both times (d). At the right of the sub-surface area designated by dots the corresponding area beneath the dark aleurone can sometimes be made out as slightly darker than the remaining part of the seed. One must be careful to guard against an optical illusion due to a shadow from the dark surface above. In spite of this possibility and the danger of a too vivid imagination it is certain that these areas as expected are sometimes darker than usual.

VARIEGATED CHANGES

In the seeds just described the region below the colorless surface is frequently variegated waxy and normal. Apparently *Wx* does not accompany *C* at the first unequal mitosis but is lost frequently in subsequent divisions. The variegated tissue in cross section covers the area that has lost the *C* gene, judging from similar mosaics where both *C* and *Wx* are

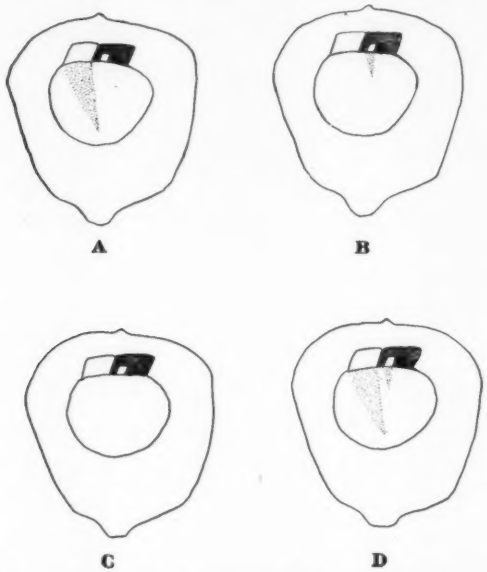
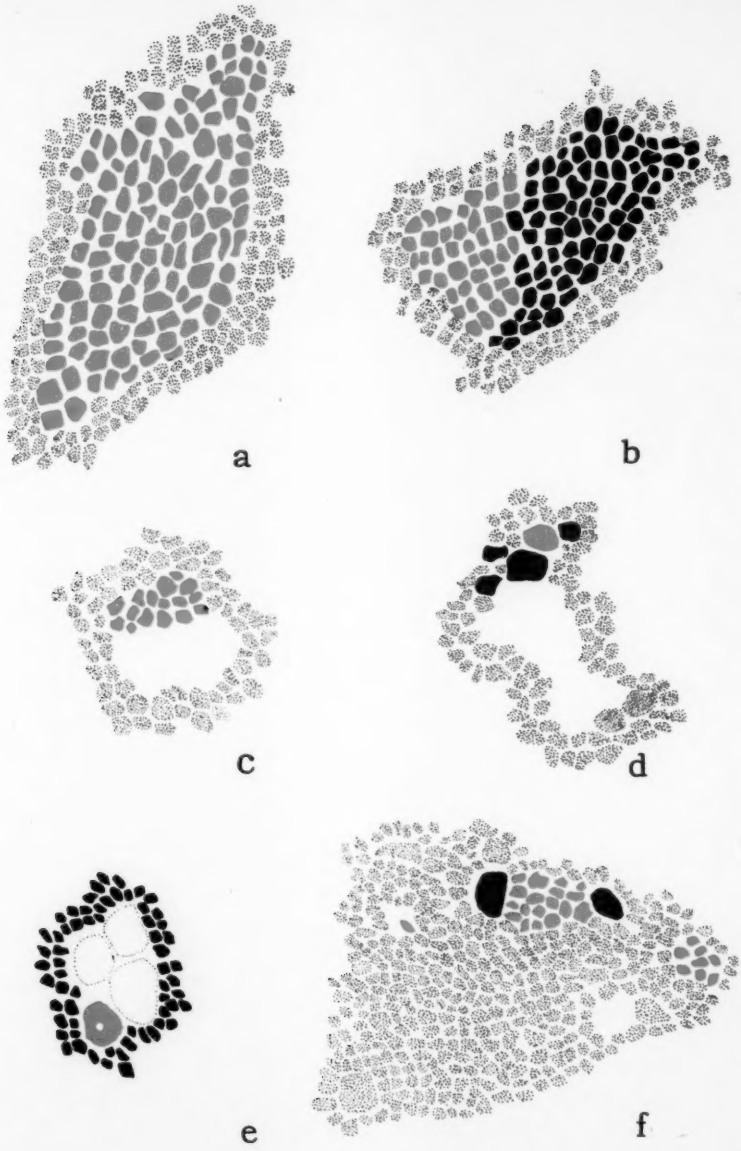


FIGURE 11.—Diagram showing the behavior of linked genes in primary and secondary twin spots in the aleurone and endosperm of maize seeds. The cross-hatched area represents the first doubling of the *C* gene, the solid black the second doubling. The dotted area represents waxy tissue.

lost the first time. *When one gene is lost, other genes may be removed in subsequent mitoses with a much higher frequency.* Variegated areas in great variety and complexity are also seen on the surface of seeds heterozygous for *C* or *Pr*. Clearly delineated areas have within their borders mixtures of white, light and dark cells in one case and red and dark cells in the other

EXPLANATION OF PLATE I

Camera lucida drawings of changes in color and size of aleurone cells occurring naturally on maize seeds heterozygous for *C* and *Pr*. (a) unpaired *pr* mosaic. (b) paired red and dark cells due to shift of *Pr* gene. (c) inter-chromosomal paired mosaic showing loss of *C* in the colorless part and loss of *Pr* in the red part. (d) a change to colorless, light red (dotted), dark red (solid), dark and a large dark cell. (e) paired red and colorless giant cells. (f) giant cells with no change in color, a giant cell paired with colorless cells, a red celled area, a red cell paired with colorless cells, and red cells paired with giant dark cells. ×90.


a
b
c
d
e
f

or all together. Rarely this variegation extends over the entire surface of the seed. Sometime during development a change has occurred such that the normal mechanism of mitosis is deranged. After this has happened gene shifts and losses occur with abnormally high frequency. This altered tissue grows along with normal tissue but is often irregular in arrangement of cells and may grow at a slower or faster rate.

THE TRANSMISSION OF CHROMOSOME INSTABILITY

When the seeds classified in tables 1 and 2 were planted and the second generation seeds self-pollinated, about the same kind of mosaics were found in the heterozygous seeds where dominant genes were brought with the pollen. The majority of these light colored seeds had unpaired and paired color changes in about the same frequency as the first generation. But a few seeds on several ears examined had a very high frequency of mosaics. In some cases twin spots, and twin spots within twin spots, were found all over the surface of the seed. Some kind of recombination had taken place permitting an unusually high rate of mosaicism. This is evidence that chromosomal stability is influenced by gene recombination since the cytoplasm is presumably the same for all seeds on one ear.

SOMATIC TRANSLOCATION

In tables 1, 4, and 8 are listed seeds heterozygous for both *C* and *Pr*. Among these are 28 red and white paired mosaics in a total of 6307 seeds. In this lot there are 2889 unpaired white spots and 632 unpaired red spots. One in every two seeds has a *c* mosaic and one in about ten a *pr* mosaic. Therefore one in twenty seeds should have both red and white mosaics on the same seed. But the chance that these two mosaic areas should include adjacent cells on the assumption of independent but simultaneous origin is extremely remote. There are something more than 100,000 aleurone cells on the surface of average sized seed in this family. The two changes would have to occur in two adjacent cells at about the same time and remain together until maturity. A red and white paired mosaic is shown in plate 1c. The similarity in size and shape is strong evidence for origin from a single cell. Not all such inter-chromosomal mosaics are evenly balanced in size but enough are to make plausible the assumption of a common origin.

The red and white mosaics shown in plate 1, e and f are accompanied by a change to large and to giant cells, indicating a marked cellular disturbance. In the seeds heterozygous for *C* and *Pr*, treated with X-rays after fertilization, the number of paired red and white mosaics is increased significantly over untreated material. The 46 such color variations from 560 seeds examined for waxy (table 10) do not include all the mosaics of this type that were found.

In addition to these red and white twin spots involving *C* and *Pr* in different chromosomes, paired changes are found showing losses of *Pr* and *Su*, and *Wx* and *Su* in adjoining areas. Some of these inter-chromosomal mosaics are shown in outline in figure 12. The similarity in size and shape

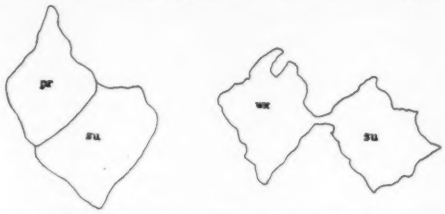


FIGURE 12.—Diagrams of inter-chromosomal mosaics in maize seeds treated with X-rays shortly after fertilization.

of the paired parts is so striking as to admit of little doubt that many of these, if not all, have a common origin from one cell at an early stage of development. In some cases white waxy spots and sugary spots were unpaired and separated on the surface but when followed down toward the

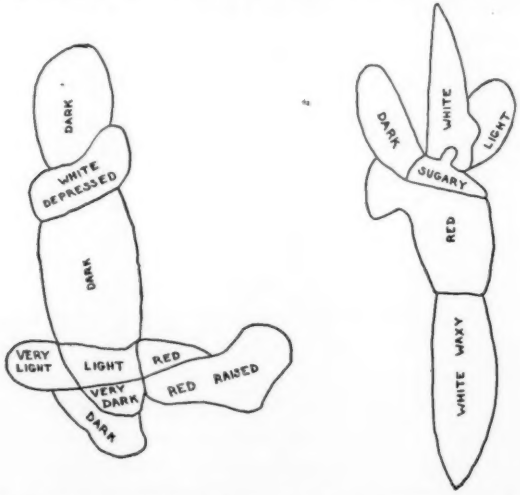


FIGURE 13.—Diagrams of surface changes in color and cell arrangement in maize seeds treated with X-rays shortly after fertilization.

center of the seed were found to come together to form a paired mosaic near the place of origin. The X-ray treated seeds also showed many extremely complex changes involving several shades of light and dark in both red and black as well as sugary, waxy, raised and depressed areas. Some of these are diagrammed in figure 13.

CHANGES IN CELL SIZE AND ARRANGEMENT

In tables 1 to 7 there are listed 266 seeds, among a total of 11,327, showing large-celled areas. This change in cell size is outlined in figure 14 and plate 1d. Large aleurone cells vary in diameter as do normal cells and

FIGURE 14.—Camera lucida drawing of part of a large celled area. Normal cells are shown at the left of the dotted line. × 160.

on the average are about twice as large. The unpigmented spaces between the colored cell centers are somewhat wider than between normal cells. Large aleurone cells are continuous with large endosperm cells underneath. They occur in seeds of all varieties examined but vary greatly in fre-

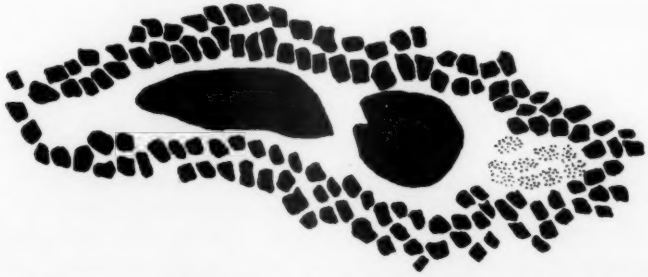


FIGURE 15.—Two giant aleurone cells paired with lightly colored cells with a border of normal cells for comparison. ×160.

quency. In area they range from single cells to about one fourth the visible surface of the seed. They are not so easy to see as color mosaics and the frequencies given in the accompanying tables do not represent all such changes that occur. Large cells are much more easily seen in some material than in other and are frequently paired with color and growth changes.

Giant cells

Large cells of various shapes pass into the giant cells shown in figure 15 and plate 1, figures e and f. Giant cells are usually associated with some kind of twin spotting, although they may stand out alone as in plate 1f. In figure 15 two large heavily pigmented areas are shown surrounded by a large amount of colorless material. At one end is a mass of material that appears to be made up of disintegrating cells almost entirely devoid of pigment. In plate 1e one large red mass encloses a smaller colorless sphere, possibly a large nucleus. With this there are three colorless masses, represented by dotted lines in the drawing, adjacent to the colored body. In plate 1f the unpaired giant cells vary by degrees from normal-sized cells and are no darker. Two heavily pigmented masses, presumably cells, are associated with a red-celled area.

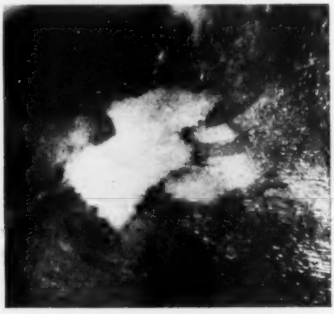


FIGURE 16.—A depressed colorless area surrounded by irregular tissue in a seed with the aleurone color composition of *C C c*. ×10.

Cell failure

All material examined has shown a small proportion of seeds with irregularities in the surface varying from round dimpled depressions to large, furrowed cavities. In some cases the pericarp is continuous over a hollow space below. In these the remains of cell walls line the cavity. In other cases the pericarp adheres to the irregularly sunken surface. When cut with a file and stained with iodine the cells immediately below and for some distance toward the center of the seed are usually colorless. Depressions can be distinguished in this way from sugary mosaics.

The failure to stain indicates the absence of starch. The cells have the usual size but shrink in drying more than normal cells. In other cases the cells break down leaving only the remains of cell walls. In 12,885 seeds examined in tables 1 to 8 there are recorded 175 depressions. An irregular depression associated with light color and failure of color is shown in figure 16.

Outgrowths

Along with depressions, sometimes on the same seed, there were found 67 raised areas where the cells have grown out beyond the remaining surface of the seed. In most cases these take the form of flat, more or less circular areas, with a well defined margin. When these areas are cut with a file and stained with iodine the cells in the raised part and for some distance below are darker than the adjoining tissue. The starch content has been changed in such a way that it absorbs more stain.

Usually the pericarp is intact, without discoloration, and there is no indication that the cells have been infected or injured. The maize ear is well protected by many soft husks. Any external mechanical injury could hardly affect a sharply defined area. Insects often do damage but these

FIGURE 17.—A spherical mass of cells growing from the surface of a maize seed. ×7.

destroy the pericarp and their action can be identified. In some cases the outgrowth is so extensive as to rupture the pericarp as shown in figure 17. Many seeds are found with the pericarp split and the endosperm bulged out. This seems to be due to a release of pressure. However, when normal seeds were tested, small punctures of the pericarp made with a sharp knife at different stages of development did not result in any outgrowing tissue.

In figure 17 the outgrowth is spherical in shape and has about the same texture as the normal part of the seed. Growth of the main mass stopped and then continued later in a smaller mass of lighter-colored cells which budded out at one side. At the base of this tumor-like structure the pericarp is discolored. Other smaller outgrowths occur nearby indicating a general region of abnormal growth. On a plant heterozygous for fasciated ear one seed was found with a similar outgrowth. In both cases the outgrowing cells are clear and translucent and give no indication of any parasitic organism. There is also no clue as to the genetic change which

may have occurred. Similar masses of cells growing in an uncoordinated manner are found in the citrus fruits.

In food storage tissue any change in cellular activity might be expected to result in a failure to accumulate reserve materials, and for that reason most abnormal growths, even where there is a large increase in the number of cells, would be seen as depressions after the seeds or fruits were matured and dried out. It is notable that tissue, growing at a faster rate than normal or after development has ceased elsewhere, has retained the ability to lay down food reserves in the same manner as normal tissue.

An example of abnormal tissue growing at the expense of normal tissue is given in figure 18. At the base of this ear four rows of kernels are aborted. As the ear developed this sterile sector widened out until at the tip it occupies nearly the entire structure and has forced the ear into an un-

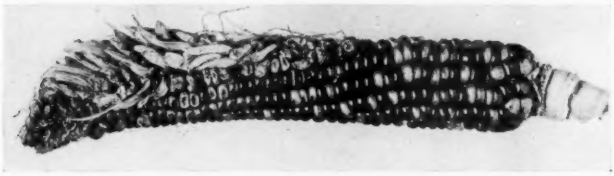


FIGURE 18.—A maize ear with a sterile sector of tissue which has grown at a faster rate than normal tissue. In this area the glumes which are normally suppressed have elongated in a freakish manner.

balanced shape. At about the middle of the ear the glumes at the base of each kernel, which are normally suppressed, elongated, and at the tip present an extremely freakish appearance.

No seeds were produced in this abnormal sector. The adjacent normal seeds were planted and two generations have been grown from them. The original ear was naturally pollinated. Later generations were selfed and out-crossed. No exact return of the abnormal condition has been observed but many ears show an irregular development of the cob with a tendency to abort some of their ovules. Some of the second generation plants with colorless seeds were naturally cross-pollinated with purple aleurone. These outcrossed seeds were found to have an enormous number of small color and growth changes in the aleurone. None of these were visible to the eye but under the microscope a most extraordinary picture was presented. A small area from one of these seeds drawn with a camera lucida is shown in plate 1f. This area, about 1mm by .5mm, has at least 10 abnormal areas of one or more cells each, consisting of large cells, giant cells, red spots, red and white paired mosaics, red and dark, and white and large paired cells. Many of the colorless spots are raised above the surrounding tissue.

This unusually high rate of mosaicism is found over the entire surface of many seeds on these ears. The same situation was also found on some of the seeds on the original ear shown in figure 18. There is no proof that this high rate of cellular aberration in the seeds has anything to do with the abnormal development of the ear but neither type of aberrancy has been found elsewhere. Further investigations are under way to study this peculiar and highly significant occurrence.

Paired growth changes

Raised areas on the surface of the seed are many times adjacent to depressions. The paired sectors may be similar in size and outline or quite

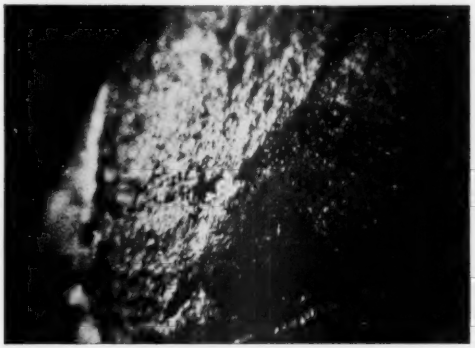


FIGURE 19.—Paired depression and outgrowth with colorless cells between. ×10.

uneven. The latter type is shown in figure 19. At the lower left corner of this mosaic area there is a deep, pit-like depression. On the margin is a small colorless area. Adjoining the colorless part there is a circular raised area that forms part of a larger triangular area that is also raised but not so high. At the upper corner of the raised area are two irregular depressions.

In some cases the raised parts are darker than normal in the aleurone and the depressed parts are normal, red or white in color. In other cases the reverse is true. There is no consistent association of color changes with growth changes. In the majority of instances no aleurone color alteration accompanies the atypical growth but the endosperm underneath stains darker and lighter than normal corresponding to the raised and depressed areas of the surface. The evidence is clear that a shift of cellular material has taken place.

CYTOLOGICAL BASIS FOR SOMATIC CHANGES

In the investigations of GUIGNARD (1901), WEATHERWAX (1923) and RANDOLPH (1936) the development of the endosperm of maize has been followed in considerable detail. After the fusion of the second male nucleus with the two polar endosperm nuclei, division proceeds rapidly until there are many free nuclei lining the embryo-sac cavity. From 128 to 256 free endosperm nuclei have been formed, according to RANDOLPH, by the time the embryo reaches the 6 to 8-cell stage, with a definite tendency for the divisions to take place in unison. Four days after fertilization cell walls have been laid down in all parts ordinarily, except in the antipodal portion. Cell formation proceeds from the outer parts toward the center filling the middle region formerly occupied by a large central vacuole. Beginning a few days after fertilization the endosperm grows in length rapidly. Increase in breadth is at first more rapid at the base. In the later stages of growth the apical part increases in volume more rapidly.

The outline of mosaic areas confirms these findings in the main. At the basal part of the seed most of the mosaics are long and narrow. At the tip they are more nearly square or circular. If segregation occurs in the early free nuclear stage the daughter cells are quite likely to become separated and therefore may not show as paired mosaics. In general, large color changes covering one fourth or more of the seed are usually single. Waxy and sugary mosaics that can be followed in endosperm tissue are roughly cone-shaped with their tips pointed toward the center or base of the seed. Cell separation in some cases occurs late in development. Mosaic areas with a mixture of dark and colorless cells have been found.

WEATHERWAX states that both mitotic and direct divisions occur, the latter being common in the later stages of endosperm development. Even in mitotic divisions he finds there is much variation from an equal distribution of chromosomes. In this short-lived food-storage material the exact quantitative division of the chromatin is not so closely guarded as in other tissues. Both the cytological and genetic evidence are in agreement on this point.

Incomplete fertilization

There is only one case on record known to the writer to indicate that there is any failure of nuclear fusion according to the early hypothesis of CORRENS and of WEBBER. A seed heterozygous for genes in more than one chromosome has been found by RANDOLPH (1932) exhibiting all the characters of the female parent in approximately one half of the seed after heat treatment. The other half showed characters brought in with the pollen. Genes would have to be lost from two and possibly three different chromosomes to produce this result if fertilization took place normally.

The part of the seed showing only maternal characters had smaller cells than the part showing the hybrid characters. RANDOLPH interprets this as a case of incomplete fertilization—one polar nucleus developing independently to produce haploid tissue, the other fusing with the second male nucleus to form hybrid diploid tissue. If this happened frequently many more seeds of this kind would be expected on the basis of independent development of parental tissue. On the other hand the expression of characters contributed by both parents in hybrid endosperms in mosaic tissue shows that fusion of the endosperm nuclei with the male nucleus regularly occurs. RANDOLPH'S case could be due to an interchange between two chromosomes at an early stage followed by the loss of both chromosomes from the resulting cells.

Mutation

Changes from a recessive aleurone color to the dominant condition in homozygous seeds have been found, (JONES 1936a) with a frequency of about 1 in 200 as compared with reverse changes in heterozygous plants. Presumably these are alterations at a definite locus that are not accompanied by any shift or loss of chromatin although proof is lacking. Similar changes from the dominant to the recessive condition might be expected with greater frequency and such variations do play a part in somatic diversity. The mutable characters described by DEMEREC (1928) are associated with an increase in crossing over. Something other than mutation as generally understood is involved in these variations as well as the variegated pericarp described by EMERSON (1917).

Non-disjunction

The failure of whole chromosomes to divide properly in heterozygous material results in the removal of dominant genes from some cells with a corresponding doubling in the other daughter cells. This would account for twin spots where all linked genes are shifted together but does not explain the many instances where linked genes are removed separately. It is also inadequate to account for the many unpaired mosaics. Lagging chromosomes that are lost from all cells would account for unpaired mosaics where all linked genes are lost simultaneously. But non-disjunction seems doubtful in view of the even growth of the two parts of paired mosaics.

Reciprocal non-disjunction, whereby homologous chromosomes are distributed to daughter cells without chromatid separation resulting from spindle fibre point segregation, would result in paired mosaics that have a balanced chromatin content and might be expected to give equal growth rates in the daughter cells. Chromatid crossing over before segregation would give waxy mosaics without color change and these have not been

observed. Without crossing over the separation of linked genes precludes this possibility as a major factor.

Deletion

Deficiencies in maize chromosomes involving terminal and internal pieces have been demonstrated both by cytological and genetic evidence by McCLINTOCK (1931, 1932) and STADLER (1933). In most cases the growth rate of the deficient cells is reduced. Deletion may account for many of the unpaired mosaics but can play no part in the paired changes.

Translocation

If pieces that are removed from some chromosomes are attached to others, either homologous or non-homologous, twin spots could result and linked genes would be separated depending upon the point of breakage. Evidence for translocation is furnished by the paired mosaics exhibiting the effects of recessive genes in different chromosomes. Many red and white paired spots have been found in untreated material and an increased frequency of such inter-chromosomal mosaics involving *C—Pr*, *Pr—Su*, *Su—Wx* has been noted in seeds exposed to X-rays shortly after fertilization. In a few cases the area of the paired parts is approximately equal but in many cases growth is decidedly at a different rate and there are also marked tissue alterations.

Paired mosaics of this kind may result from a simple translocation of a piece from one strand to another non-homologous strand followed by the loss of the augmented chromosome having two spindle fibre attachment points. This would account for the loss of dominant genes in adjacent areas assuming viability of the deficient cells. Evidence against this occurrence and in favor of a reciprocal interchange between non-homologous chromosomes is at hand in a red and white twin spot in the X-ray treated seeds. The red area on the surface showed a loss of the *Pr* gene. The colorless area resulted from the removal of *C*. This white area was also waxy. *Pr* could have been translocated to the *C Wx* chromosome or the reverse, and either increased chromosome having two attachment points could have been lost at a subsequent division. In either case the result would be a paired red and white-waxy mosaic. But the endosperm underneath the red aleurone was distinctly darker than normal when stained. This shows that something replaced the missing *Pr* gene. This was most probably the missing *Wx* gene.

MATHER (1934) X-rayed Tradescantia and found meiotic metaphase chromosomes to be extremely fragmented at four and seven days after treatment. Translocations and configurations with more than two attachment points were present. RILEY (1936) also treated Tradescantia in the

same way and found the most common abnormality at metaphase and anaphase to be fragmentation. The broken pieces ranged in size from apparently single chromomeres to pieces almost the length of a whole arm of a chromosome. Translocation, deletions, segmental interchange figures, and Z-shaped chromosomes which had two spindle fibre points were observed. After three weeks few abnormalities were noted. Evidently all interchange types with a deficient chromosome complement and large deletions were eliminated. BRITTINGHAM (1931) finds that radium produces necrotic tissue in the bud-clusters of Oenothera but no effect of this kind was seen in Tradescantia by RILEY following X-ray treatment. Actively dividing cells were as numerous as in untreated material during similar periods. GUSTAFSSON (1937) found fragments, fused chromosomes, ring chromosomes and translocations in Hordeum seeds X-rayed in the dormant condition.

The genetic evidence from maize seeds following X-ray treatment shows that nearly all possible gene losses and rearrangements result, indicating that chromosome aberrations are numerous and that interchanges between non-homologous members do take place. Many of the resulting cells divide for some time but fail to fulfil their function of storing food and some fail to live as shown by the remains of cell walls lining empty cavities.

Somatic crossing over

If reciprocal translocation can take place between non-homologous chromatids it is even more likely to involve homologous chromatids. Evidence that somatic crossing over does occur has been obtained by STERN (1936) in Drosophila who has also reviewed earlier investigations bearing on this problem. He concludes: "The mechanism of mosaic formation is not based on simple elimination of chromosomes but on processes of somatic crossing over involving two strands of a four strand group. Segregation of the four strands occurs in an equatorial, typically mitotic mode in respect to the fibre points. It leads to homozygosis of originally heterozygous genes. No reduction of number of chromatids takes place in normal cases."

MCCLINTOCK (1932) has found evidence for somatic crossing over in maize based upon cytological observations of different chromosome constitution in diverse cells of a variegated plant. The variation in size of ring-shaped chromosomes indicates an exchange of chromosome parts in somatic tissue.

In the Diptera, homologous chromosomes are together during development giving opportunity for crossing over. An association of homologous chromosomes during mitosis in plants has been observed by several investigators. The evidence is reviewed by HEILBORN (1936). In Drosophila

crossing over near the spindle fibre is more frequent in somatic cells than in germ cells. In Zea, *C* and *Wx* are shifted together in somatic cells from 45 to 51 percent of the times in the translocation stock, (table 9) and from 65 to 80 percent in the X-rayed material (table 10). The latter value is close to the germ cell linkage value (74 percent). Both experiments are based on unusual material. Linkage values from normal untreated plants may show a different result.

Triploid tissue is not satisfactory to prove or disprove somatic crossing over. For this purpose it is desirable to have linked dominant genes contributed by both parents so that a dominant gene may be removed from one chromosome and replaced by its recessive allele. In triploids this result is ordinarily masked by the presence of another dominant in the third homolog. In diploid tissue paired mosaics showing the effect of linked genes brought into visible expression in adjacent areas should give critical evidence. Pericarp color alleles may be useful for this purpose. A red cob, colorless pericarp plant crossed by one with a white cob, red pericarp has color in both parts. Mosaic patches of colorless seeds should be underlaid by colored cob and adjacent areas of colored seeds should have colorless cob. This situation could also result from an interchange between homologous chromosomes but not at homologous loci. But a chromosome unbalance of this kind would show in later generations.

For the present, somatic crossing over is an attractive hypothesis that seems to fit many of the observed facts. It is an addition to previous theories and does not replace them. Like all of the hypotheses previously advanced it may yet, with these, prove to be inadequate to account for all of the facts. The colorless areas in homozygous seeds are not readily explained by any cytological mechanism now known and the secondary twin spots are not easily accounted for on the basis of somatic pairing and interchange. The reversal of recessive changes to the original dominant condition can not be the result of somatic crossing over.

A GENETIC INTERPRETATION OF ATYPICAL GROWTH

The large cells and giant cells previously described are most probably due to an increase in chromosome number. WINKLER (1916) in Solanum, TUPPER and BARTLETT (1916) in Oenothera, SINNOTT and BLAKESLEE (1922) in Datura, RANDOLPH (1932) in Zea, ATABEKOWA (1937) in Pisum, MÜNTZING (1937) in Dactylis show increased cell size to be associated with a multiplication of chromosomes. SASS (1937) has examined maize seedlings injured by mercuric dust treatment and found abnormal cells differing greatly in size with corresponding minute and giant nuclei varying in chromosome number. STEIN (1936) finds a large increase in chromosome number associated with giant cells and abnormal growth in a fasciated

Antirrhinum resulting from radium treatment. Large abnormal cells are particularly noticeable in the growing points of stems and roots.

No chromosome counts have been made on the naturally occurring large cells in maize aleurone but they may be assumed to be polyploid. The giant cells of varying size and color represent large accumulations of chromosomes. Giant cells are usually paired with colorless areas, depressions or outgrowths and show clearly that an irregularity in mitosis has taken place. The outgrowths that rupture the pericarp and extend above the surface of the seed in a spherical mass can not be attributed to me-

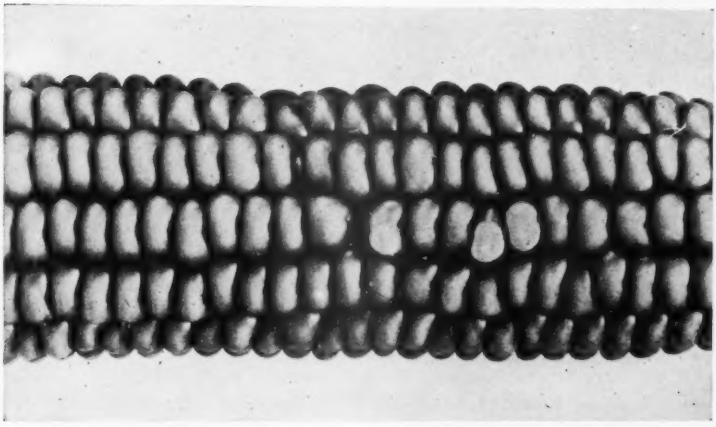


FIGURE 20.—White, opaque outgrowths on five adjacent seeds showing a maternal influence on atypical growth.

chanical injury or infection. They seem to be extremes in a series of growth changes many of which clearly start with an unequal mitosis and are accompanied by the removal of known genes from some cells.

Maternal influence upon abnormal growth

Outgrowths have been found on adjacent seeds of maize in a considerable area indicating some common causal factor in the maternal tissue from which the seeds grew as shown in figure 20. LITTLE (1933) has given evidence that the transmission of tumors, in certain strains of mice, is almost exclusively through the females. Males from high tumor lines rarely transmit the disease. Other strains, however, do transmit through the males. FEDERLEY (1936) reports moths that have tumors in the males but transmission is only through females. All of the males are affected and all of the females are carriers.

From the evidence in maize there is a maternal control of chromosome

stability. This operates through some part of the mechanism of cell division that is transmitted by the egg. Various strains of maize differ widely in the frequency of mosaics. Reciprocal crosses give significantly different frequencies of large-celled areas and growth disturbances. Maize is not the best material to test this point on account of the unequal chromosome contribution from the two parents but the evidence at hand is more than an indication that there is a maternal influence on the regularity of mitosis.

Any interpretation of atypical growth must take into consideration the well-known fact that fasciations and galls in plants may regenerate normal tissue. Lateral branches from fasciated stems and adventitious buds from many gall formations, when removed from the host, produce normal plants. Obviously no genetic change has taken place in the meristematic cells taking part in reproduction. It has been suggested (JONES 1937) that the change should be looked for in those cells that produce growth stimulating substances. An abnormal disturbance in the balance of plant hormones could be manifest in other parts of the plant in tissues that are not genetically changed. The giant cells in the growing points of the fasciated Antirrhinum (STEIN 1935) has much significance in this connection. Galls often appear simultaneously in many parts of the same plant and indicate some general abnormal stimulus acting over a large area. BROWN (1924) illustrates apple tree galls, where no infectious organism has been found, occurring at regular intervals on one branch. In this case latent buds or growing centers over a considerable area have been forced into active cell division. A similar situation exists in animals where diffusible substances produced at one place have a profound effect in other parts of the body.

Practically all of the abnormal growths in plants such as galls, witches' brooms, and fasciations, that are induced by parasitic organisms or other biological, physical or chemical agencies, have their counterparts in inherited variations. In these no external influence can be detected and such abnormalities are transmitted by seed and vegetative propagation and may be recombined with known genetic characters. In both animals and plants, therefore, external agencies can do little, if any, more than what may occur spontaneously in the organism itself. Any interpretation of these many puzzling and conflicting facts must take into consideration the mechanism of cell division. When normal mitosis is interfered with by external agents or inherited deviations from normal, essential growth-regulating substances originating in the cell are lost or shifted during development in such a way that some cells, deprived of these substances or receiving them in excess, change their usual activities and regulatory powers. Proof of this has been given. This deviation from normal is not dependent upon visible derangement of the mitotic structure since

it has been shown for both animals and plants that whole chromosomes, or parts of chromosomes, with mutant genes or small deletions may be substituted for normal members in somatic cell division by crossing over. But the departure from normal growth may become wider, in succeeding stages, as more and more essential genes are lost with the increasing irregularity of the mitotic mechanism, as has been shown here, until finally the cells die or lose, in varying degrees, their ability to coordinate growth with the other parts of the organism.

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SUMMARY

Previous hypotheses attempting to account for genetic changes during development are reviewed and it is shown that incomplete fertilization, mutation, non-disjunction and deletion are inadequate to account for all of the observed facts.

Paired changes, visible in adjacent areas, previously noted in pome and citrus fruits and in maize pericarp, have been found to be frequent in maize aleurone and endosperm, exhibiting the result of a shift of known color and texture genes.

More than one dominant allele may be lost or shifted from homologous chromosomes at the same time.

The *C* aleurone color gene is lost or shifted with a higher frequency when it is in a chromosome with a translocated piece attached at the opposite arm than it is in normal plants.

Linked genes, *C* and *Wx*, are lost or shifted together or separately. Their linkage is the same or less than that measured by germ cells.

Seeds treated with X-rays shortly after fertilization show a marked increase of inter-chromosomal mosaics in adjacent areas.

The dark part of a paired aleurone color mosaic may revert to normal, to colorless or may become still darker. Some of these secondary changes

are paired alterations. Linked genes may shift together or separately in these secondary changes.

Tissues that have lost one or more genes are highly unstable and may show frequent losses of other genes in subsequent cell generations.

Chromosome stability, as measured by the frequency of mosaics, varies widely in different families and from one generation to the next in the same family.

Reciprocal gene exchanges occur between non-homologous chromosomes. The evidence indicates that there is also an interchange between homologous chromosomes during development, but whether this is somatic crossing over at homologous loci or some form of unequal translocation, remains to be determined.

Changes in cell size and arrangement occur alone or may accompany changes in color and texture. These tissue alterations result in depressions and outgrowths which are sometimes paired.

Atypical growth is considered to be the result of the removal from, or concentration in, certain cells of essential growth-regulating substances brought about by unequal mitosis, with or without visible alteration of the chromosomes. This unequal mitosis may be induced by external agencies or by inherited defects of the mechanism of cell division transmitted in part through the female parent.

LITERATURE CITED

ATABEKOWA, A., 1937 Über die Bildung polyploider Sätze in somatischen Zellen. Genetica **19**: 105–133.

BRIDGES, C. B., 1914 Direct proof through non-disjunction that the sex-linked genes of Drosophila are born by the X-chromosome. Science **40**: 107–109.

BRITTINGHAM, W. H., 1931 Variations in the evening primrose induced by radium. Science **74**: 463–464.

BROWN, N. A., 1924 An apple stem-tumor not crowngall. J. Agric. Res. **27**: 695–698.

CASTLE, W. E., 1935 The Brahma-Plymouth Rock mosaic. J. Hered. **26**: 66.

COLLINS, G. N., 1913 Mosaic coherence of characters in seeds of maize. U. S. Dept. Agric. Cir. **132**: 19–21.

COLLINS, J. L., 1936 A frequently mutating gene in the pineapple *Ananas comosus* (L.) Merr. Amer. Nat. **70**: 467–476.

CORRENS, C., 1901 Bastarde zwischen Maisrassen mit besonderer Berücksichtigung der Xenien. Bibliotheca Botanica **53**: 1-161.

DAHLGREN, K. V. OSSIAN, 1927 Eine Sektorialchimäre vom Apfel. Hereditas **9**: 335–342.

DEMEREC, M., 1928 Mutable characters of *Drosophila virilis*. I. Reddish-Alpha body character. Genetics **13**: 359–388.

EAST, E. M., and HAYES, H. K., 1911 Inheritance in maize. Conn. Agric. Exper. Sta. Bull. **167**.

EMERSON, R. A., 1915 Anomalous endosperm development in maize and the problem of bud sports. Z. i. A. V. **14**: 241–259.

1917 Genetical studies of variegated pericarp in maize. Genetics **2**: 1–35.

1921 Genetic evidence of aberrant chromosome behavior in maize endosperm. Amer. J. Bot. **8**: 411–424.

EMERSON R. A., BEADLE, G. W., and FRASER, A. C., 1935 A summary of linkage studies in maize. Cornell Univ. Agric. Exper. Sta. Mem. **180.**

EYSTER, W. H., 1924 A genetic analysis of variegation. Genetics **9:** 372–404.

1925 Mosaic pericarp in maize. Genetics **10:** 179–196.

FEDERLEY, H., 1936 Sex-limited hereditary cancer in lepidopterous larvae. Hereditas **22:** 193–216.

FROST, H. B., 1926 Polyembryony, heterozygosis and chimeras in citrus. Hilgardia **1:** 365–402.

GUIGNARD, L., 1899 Sur les anthérozoides et la double copulation sexuelle chez les végétaux angiospermes. Rev. génér. d. Bot. **11:** 129–135.

1901 La double fécondation dans le maiz. J. d. Bot. **15:** 37–50.

GUSTAFSSON, A., 1937 The different stability of chromosomes and the nature of mitosis. Hereditas **22:** 281–335.

HEILBORN, O., 1936 The mechanics of so-called secondary association between chromosomes. Hereditas **22:** 167–188.

JONES, D. F., 1935 Somatic segregation due to hemizygous and missing genes and its bearing on the problem of atypical growth. Proc. Nat. Acad. Sci. **21:** 90–96.

1936a Mutation rate in somatic cells of maize. Proc. Nat. Acad. Sci. **22:** 645–648.

1936b Segregation of color and growth-regulating genes in somatic tissue of maize. Proc. Nat. Acad. Sci. **22:** 163–166.

1937 Somatic segregation in relation to atypical growth. Proc. Amer. Phil. Soc. **77:** 411–416.

LITTLE, C. C., 1933 The existence of non-chromosomal influence in the incidence of mammary tumors in mice. Science **78:** 465–466.

MCCLINTOCK, B., 1931 Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agric. Exper. Sta. Bull. **163:** 1–30.

1932 A correlation of ring-shaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. **18:** 677–681.

1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellf. mik. Anat. **19:** 191–237.

MATHER, K., 1934 The behavior of meiotic chromosomes after X-irradiation. Hereditas **19:** 303–322.

MÜNTZING, A., 1937 The effects of chromosomal variation in Dactylis. Hereditas **23:** 113–235.

NAWASCHIN, S., 1899 Resultate einer Revision des Befruchtungsvorgangs bei *Lilium Martagon* und *Fritillaria tenelle*. Bull. d. l'Acad. Imp. d. Sciences de St. Petersbourg **9.**

RANDOLPH, L. F., 1932 Some effects of high temperature on polyploidy and other variations in maize. Proc. Nat. Acad. Sci. **18:** 222–229.

1936 Developmental morphology of the caryopsis in maize. J. Agric. Res. **53:** 881–916.

RILEY, H. P., 1936 The effect of X-rays on the chromosomes of *Tradescantia gigantia*. Cytologia **7:** 131–142.

ROBERTS, E. and QUISENBERRY, J. H., 1935a A Brahma-Plymouth Rock mosaic. J. Hered. **26:** 11–14.

1935b. The Brahma-Plymouth Rock mosaic. J. Hered. **26:** 114.

SASS, J. E., 1937 Histological and cytological studies of ethyl mercury phosphate poisoning in corn seedlings. Phytopathology **27:** 95–99.

SHAMEL, A. D., SCOTT, L. B. and POMEROY, C. S., 1918 Citrus fruit improvement: a study of bud variation in the Valencia orange. U. S. Dept. Agr. Bull. **624:** 1–120.

SINNOTT, E. W. and BLAKESLEE, A. F., 1922 Structural changes associated with factor mutations and with chromosome mutations in Datura. Proc. Nat. Acad. Sci. **8:** 17–19.

STADLER, L. J., 1933 On the genetic nature of induced mutations in plants. II. A haplo-viable deficiency in maize. Missouri Agric. Exper. Sta. Res. Bull. **204.**

STEIN, E., 1935 Zur Genetik, Histologie und Zytologie einer neuen, durch Radiumbestrahlung erzeugten krebsigen Entartung von *Antirrhinum majus* (Aus Gengruppe B) Z. i. A. V. **70:** 525–531.

1936 Die Doppelchromosomen im Blütenbezirk der durch Radiumbestrahlung erzeugten Mutante "cancroidea" von *Antirrhinum majus* (Somatische Chromosomen-Reduktion). Z. i. A. V. **72:** 267–286.

STERN, C., 1936 Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21**: 625–730.

TUPPER, W. W. and BARTLETT, H. H., 1916 A comparison of the wood structure of *Oenothera stenomeres* and its tetraploid mutation *gigas*. Genetics **1**: 177–184.

WEATHERWAX, P., 1923 The story of the maize plant. Chicago: Univ. of Chicago Press.

WEBBER, H. J., 1900 Xenia, or the immediate effect of pollen in maize. U. S. Dept. of Agric. Bull. **22**: 1–44.

WINKLER, H., 1916 Ueber die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeit. f. Bot. **8**: 417–531.

CHROMOSOME BEHAVIOR AND NUCLEAR DEVELOPMENT IN TRADESCANTIA*

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A COMPARISON of microsporogenesis and microspore development in a triploid Tradescantia has shown significant deviations from the normal diploid behavior. Occasional dicentric chromatids at meiosis are found in diploid Tradescantias, but inversion crossovers in the triploid are much more frequent. An analysis of microspore development in a triploid Tradescantia shows that chromosome number has a direct effect on cell size and rate of nuclear development.

I. HETEROZYGOUS INVERSIONS

The triploid used in this work is presumably an auto-triploid of *Tradescantia bracteata* Small. The general behavior and distribution of the meiotic chromosomes in this plant have been described by KING (1933), and a triploid form of *Tradescantia "virginiana* L. (U. S. A.) var. brevicaulis" has been described by DARLINGTON (1929). In these triploids the chromosomes were usually associated as trivalents; but bivalents and univalents were also found. As in many triploids, the chiasma frequency per unit of pairing length was greater than in the diploid forms.

An analysis of 25 pollen mother cells of the triploid Tradescantia showed a total of 116 trivalents, 35 bivalents, and 32 univalent chromosomes. If only homologous chromosomes pair, the total number of trivalents and bivalents should not exceed six, but in one cell there was an extra bivalent, presumably formed by chromosomes only partially homologous. No other aberrations in pairing were observed. The average chiasma frequency for bivalents was 2.0, of which 29 percent were interstitial; while for the trivalents the average chiasma frequency was 2.4, of which 17 percent were interstitial.

At meiotic anaphase the chromosomes are distributed at random to the poles, with the exception of the lagging univalents. An analysis of 74 late anaphase figures which included no lagging chromosomes, showed the following distribution.

TABLE 1

Chromosome distribution at 1st anaphase.

	9–9	8–10	7–11	6–12	
Frequency	37	30	6	1	$X^2=3.5$
Theoretical	35	26	11	1	P= .18

* A part of the cost of the accompanying figures is met by the Galton and Mendel Memorial Fund.

Two types of abnormalities were observed at the first meiotic anaphase. Lagging chromosomes were frequent, and there were many fused chromatids and chromatid fragments. The lagging chromosomes will be considered later, in the description of microspore development. Fused chromatids and fragments at meiosis are found in many species of plants and animals, but not until McCLINTOCK (1933) showed their relation to crossing over in heterozygous inversions, did other cytologists realize their significance (MÜNTZING 1934, RICHARDSON 1936, DARLINGTON 1937, SAX 1937).

About 18 percent of the pollen mother cells of the triploid Tradescantia show fused chromatids at meiotic anaphase. Fused chromatids may occur with no visible fragment, but chromatid fragments were always accompanied by fused chromatids. In the triploid two homologous chromosomes connected by a fused chromatid may pass to opposite poles or to the same pole, so that a complete analysis requires figures in which all anaphase chromosomes can be differentiated. In 123 such figures 101 showed no fused chromatids, 15 showed fused chromatids between poles, and 7 showed fused chromatids at one pole. In only one cell was there more than one fused chromatid. In the 22 cells with fused chromatids there were 12 with no visible fragment, 7 with a small fragment, and 3 with a large fragment chromatid. At late anaphase an analysis of 67 cells with fused chromatids between poles showed 38 with no visible fragment, 17 with a small fragment no longer than the diameter of the chromatid, 9 with frag-

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DESCRIPTION OF PLATE 1

Photographs of pollen mother cells and microspores from a triploid *Tradescantia bracteata*. Aceto-carmine preparations. ×1000.

FIGURE a. Metaphase of first meiotic division. Five trivalents, one bivalent, and one univalent.

FIGURE b. Meiotic anaphase with two lagging univalents.

FIGURE c. Late anaphase with dicentric chromatid, an acentric fragment at the right (somewhat below focal plane), and a dividing univalent.

FIGURE d. Meiotic anaphase with dicentric chromatid retarding division of bivalent, a small fragment, and a lagging univalent.

FIGURE e. Late anaphase showing retarded dicentric chromatid. The normal sister chromatids of this bivalent have passed to the poles.

FIGURE f. Early anaphase in *T. gigantea* (X-rayed egg cell) showing dicentric chromatid and release of the acentric fragment.

FIGURE g. Microspore chromosomes with chromatids separated even at the centromeres at very late prophase.

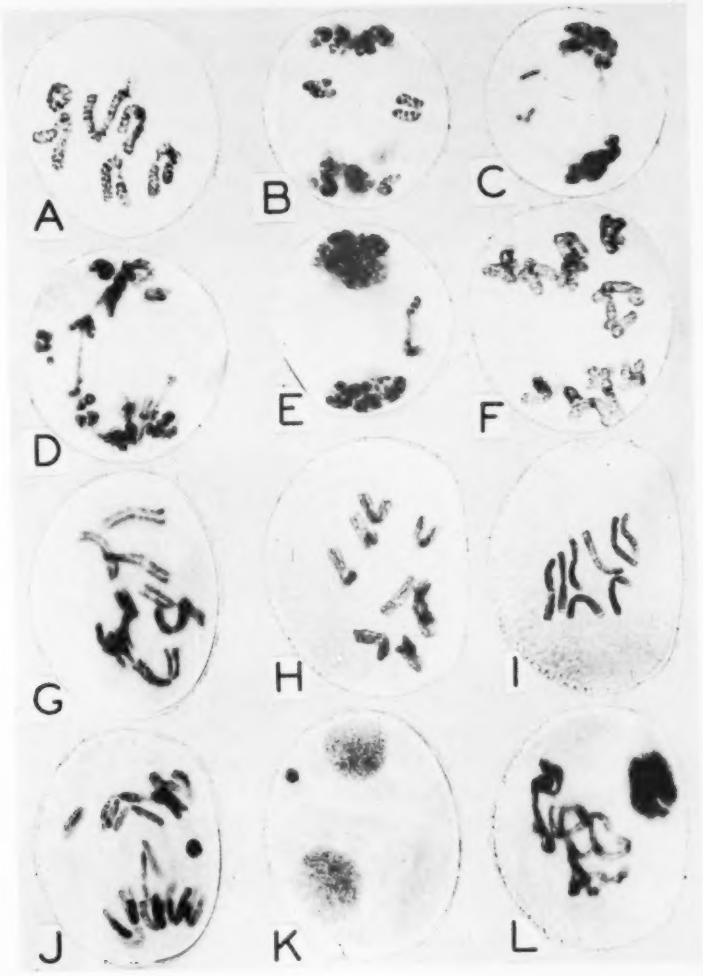
FIGURE h. Microspore chromosomes at metaphase. Chromosomes short and thick.

FIGURE i. Long, slender types of chromosomes in microspore at metaphase.

FIGURE j. Aberrant orientation of spindle in microspore nucleus division. A dicentric chromatid towards lower pole and a degenerated "lost" univalent.

FIGURE k. Failure of nuclear differentiation following disturbed polarity of the axis of nuclear division.

FIGURE l. A microspore with early differentiation of the generative nucleus, but with the "tube nucleus" at mid-prophase.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ments about a third of the length of the chromosome arm, and 3 with large fragments nearly as long as a chromosome arm. No fragments were observed which were longer than a normal chromosome arm, although a short inversion near the centromere should give such fragments.

The various types of figures resulting from crossing over in chromosomes heterozygous for inverted segments have been described by RICHARDSON (1936). In the triploid Tradescantia almost all the dicentric chromatids appear to be derived from a single crossover in the inverted segment. The dicentric chromatid usually forms a bridge between the poles at late anaphase, or connects two homologues at one pole. The dicentric chromatid may be broken, but it usually persists and retards the passage of the associated homologues to the poles (Plate 1, fig. d). At late

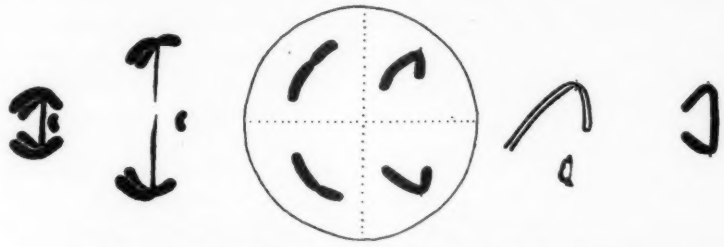

FIGURE 1.—The origin of dicentric chromatids found in the microspore.

Meiosis Tetrad Microspore

anaphase the retarded homologues connected by the fused chromatid may divide equationally and the monocentric chromatids pass to the poles, leaving the dicentric chromatid between the poles (fig. e). STURTEVANT and BEADLE (1936) have postulated such a behavior of the chromosomes to explain the genetic behavior of crossovers in heterozygous inversions in Drosophila. The dicentric chromatids which form bridges at first anaphase may persist at the second division, and those which connect homologues at one pole in the first division may form additional bridges at the second division.

Chromatid bridges are found occasionally in the mitosis of the microspore (fig. j). Such dicentric chromatids are apparently derived from dicentric chromatids broken at meiosis. The broken ends of sister chromatids appear to fuse to form a new dicentric chromatid at the first postmeiotic division (fig. 1).

This behavior is essentially the same as that found in certain ring chromosomes in Zea (MCCLINTOCK 1932), and indicates that the normal end of a chromosome has properties not found in interstitial loci. According to DARLINGTON (1937), the broken bridges of dicentric chromatids do not

show a subsequent fusion of the ends of sister chromatids; but UPCOTT (1937) has described chromatid bridges in the division of the microspore nucleus in Tradescantia and in Hyacinthus. Although these bridges are attributed to "spontaneous chromosome changes," they may be caused by breaks in dicentric chromatids at meiosis. If the fusion of sister chromatids at broken ends is a general phenomenon, permanent changes in chromosome morphology cannot be effected by terminal deletions or terminal inversions.

The frequency of dicentric chromatids in triploid Tradescantias is much greater than it is in the closely related diploid species. We have examined two normal plants of *T. gigantea* Rose, and one which was derived from an X-rayed egg cell, a clone of *T. paludosa* Anderson and Woodson, a tetraploid form of *T. canaliculata* Raf., and an F_1 hybrid between *T. canaliculata* × *T. paludosa*. The number of anaphase figures examined and the number and percentage of fused chromatids per cell are shown in table 2.

TABLE 2

SPECIES	NUMBER OF CELLS	FUSED CHROMATIDS	
T. gigantea 1	200	1	0.5%
T. gigantea 2	62	3	4.8%
T. gigantea (X-rayed)	235	20	8.5%
T. paludosa	406	14	3.4%
T. canaliculata	50	2	4.0%
T. canaliculata × *paludosa* F_1	200	1	0.5%
T. bracteata (3n)	545	89	16.3%

The frequency of pollen mother cells with dicentric chromatids resulting in crossovers in heterozygous inversions is from less than 1 percent to nearly 5 percent in the diploids examined. The increase in dicentric chromatids in the plant produced by a normal pollen grain and an X-rayed egg cell is presumably caused by additional inversions induced by X-ray treatment. In the triploid, however, the great increase in dicentric chromatids at meiosis is probably not caused by a greater number of inversions per chromosome than are present in the closely related diploids, but is presumably related to the type of chromosome association at meiosis. In a diploid the pairing of normal segments would tend to prevent homologous association of a heterozygous inversion, unless the inversion were large; but in a triploid the alternate association of any two segments would facilitate a homologous association of the regions heterozygous for inversions. Crossovers per unit of pairing length are more frequent in the triploid, which would also increase the number of dicentric chromatids per cell. If inversions were more frequent near the centromeres, where chias-

mata are seldom formed, an increase in interstitial chiasmata would produce an increase in fused chromatids; but the percentage of interstitial chiasmata is reduced in the triploid. The frequency of inversion bridges with no visible fragments also indicates that the inversions are very short and close to the ends of the chromosome arms.

It is difficult to account for the fact that more than half of the dicentric chromatids are not accompanied by visible fragments. The most plausible explanation is that such dicentric chromatids result from crossovers in very small heterozygous inversions very near or at the ends of the chromosome arms, and that the fragment is too small to be seen. But terminal inversions are rare (KAUFMANN 1936); and if sister chromatids fuse at broken ends, a truly terminal inversion could not survive. Moreover, very small inversions would tend to pair in a non-homologous association. The production of chiasmata very near the ends of the chromosomes would also present difficulties in interpreting crossing over on any torsion theory of crossing over, because the ends would be free to rotate. Crossing over does occur very near the ends of chromosomes, however, and presumably the crossovers in heterozygous inversions at such loci in Tradescantia release fragments too small to be seen at metaphase. The nature of known translocations seems to rule out the possibility that true terminal fusions are involved in the production of the dicentric chromatids with no visible fragments.

The lagging chromosomes at meiosis in the triploid Tradescantia include univalents which usually divide equationally, and chromosomes which are dicentric or are retarded by the chromatid bridge. At the time the tetrads are formed, there is an average of 0.44 chromosomes which lie free in the cytoplasm. They assume a more or less spherical form, as a rule, and do not divide. The loss of chromosomes by lagging at meiosis accounts for some of the pollen sterility; but many of the lost chromosomes must be duplicated in the nucleus because these unorganized chromosomes are found at the time of the first post-meiotic division in the pollen grain (Plate 1, figs. j and k). At this time the average frequency is .30 per microspore. Evidently the loss of chromosomes in the cytoplasm is not the sole cause of undeveloped pollen grains.

II. THE EFFECT OF CHROMOSOME NUMBER AND CHROMOSOME BALANCE ON MICROSPORE DEVELOPMENT

It has long been known that the doubling of the chromosome number results in a corresponding increase in cell size. In the experimental production of polyploidy, and presumably in nature, the doubling of the chromosome number is effected by the suppression of both nuclear and cell division, so that both chromosome number and cytoplasmic volume

are increased simultaneously, and the ratio is maintained in subsequent cell generations.

The formation of the cell walls at the end of meiosis in the triploid, presumably produces microspores of equal size, although the chromosome number may vary from 6 to 12. We have compared the variability in size of the newly-formed microspores in normal diploids with those of the triploid. A similar comparison was made at the time of the nuclear division, at which time the microspores had doubled in volume. The data are shown in table 3.

TABLE 3

Size and variability of microspores.

		LENGTH IN MM (×650)	
	N	M	CV
T. paludosa (2n)			
(1) Just released	46	19.9±.07	3.5±.24
(2) Nuclear division	30	25.0±.11	3.6±.31
T. bracteata (3n)			
(1) Just released	42	25.2±.09	3.3±.24
(2) Nuclear division	192	30.3±.10	7.0±.24

The microspores of the diploid have approximately the same cytoplasmic volume and the same chromosome number. Although the volume is approximately doubled between the time they are released from the tetrad and the time of nuclear division, the variability in size is not increased. This variability can be attributed to normal variation and random sampling. The young microspores of the triploid are about twice as large as those of the diploid at the corresponding stage, but the variability is no greater. These microspores also double their size by the time the nuclei divide, but at this stage the variability is greatly increased. The increased variability of the microspores of the triploid can be attributed to variations in chromsome number and chromosome balance. This relationship is shown in table 4.

TABLE 4

Tradescantia (3n) *microspores.*

		LENGTH IN MM×650											
		25	26	27	28	29	30	31	32	33	34	35	N	CV
CHROMOSOME NUMBER	6		1		3	1	1						6	4.3±.8
	7		2	1	5	6	3	2	1				20	5.2±.5
	8	2	1	5	12	14	9	8	5	5	1	1	63	7.7±.6
	9			3	5	9	13	9	11	3	4	2	59	7.1±.6
	10				2	3	7	4	6	6	3	3	34	5.4±.4
	11						1	1	2	4	1		9	4.6±.7
	12								1				1	
		2	4	9	27	33	34	24	26	18	9	6	192	7.0±.2

r=.41±.04 n=.47±.04

There is a direct relation between chromosome number and microspore size in the triploid Tradescantia. The correlation is not high because of the great variability in size of microspores in each array, but the regression is linear, as shown by the fact that the correlation ratio ($n = .47 \pm .04$) is only slightly greater than the correlation coefficient ($r = .41 \pm .04$). The variability in size of microspores is greatly increased by differences in chromosome balance, and the variability decreases as the chromosome number approaches a normal balance of 6 or 12. Evidently different chromosome combinations, even where the number is the same, have different effects on cell growth. Microspores with eight chromosomes may be smaller than the average size of haploid microspores. It is clear that both chromosome number and chromosome balance have a direct and immediate effect on the growth of the microspore.

The chromosome number of the microspores of the triploid approaches a normal frequency distribution, but is skewed because of the loss of chromosomes at meiosis. Apparently any combination of chromosomes is able to function in microspore development through the division stage, as long as the six basic chromosomes are present.

The rate of nuclear development and nuclear division also appears to be correlated with chromosome number and chromosome balance. The average chromosome number was lower in microspores from anthers in which most of the microspores were still in the prophase stage, than in those from anthers in which most of the microspores had undergone nuclear division. The differences are shown in table 5.

TABLE 5

Chromosome number of microspores.

	N	M	CV
Early divisions	50	$8.4 \pm .11$	14.3 ± 1.0
Late divisions	42	$8.9 \pm .07$	7.9 ± 0.7

The early divisions had a much greater variability in chromosome number, owing to the tendency for microspores with numbers approaching the normal balance of 6 or 12 to divide early. Apparently both chromosome number and chromosome balance have an effect on the rate of nuclear development.

Another effect of chromosome number and balance is shown in the development of the individual chromosomes. Considerable difference was found in the degree of chromosome contraction in different microspores at the time of division. In some cells the chromatids were well separated, even at the centromere, at early metaphase (fig. g). In other cells the chromosomes were relatively long but showed little separation of sister

chromatids (fig. i). Many cells contained metaphase chromosomes which were thick and short (fig. h). The variation in chromosome length appears to be greater in the microspores of the triploid (C.V. = 12.1 ± 2.0) than in those of normal diploids (C.V. = 7.0 ± 1.1). The variation is caused by differences in the degree of contraction of the chromatids which, in turn, appears to be conditioned largely by variation in chromosome balance. Microspores with the same chromosome number may vary considerably in the length of their chromosomes, so that different chromosomes reduplicated seem to have different effects on chromosome development.

Several other chromosome aberrations were observed in the microspores of the triploid Tradescantia. Occasionally the chromosomes appear to be released from the nuclear membrane at an early stage in develop-

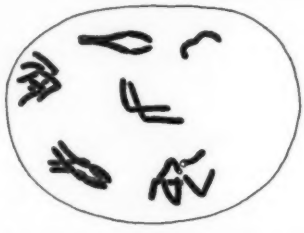

FIGURE 2.—Microspore chromosomes showing failure of orientation on spindle, precocious division of sister chromatids, and association of homologous chromosomes. Camera lucida drawing. ×1000.

ment, and at the time of chromatid separation they are not organized on a spindle. Two sister chromatids may separate precociously, and occasionally one or both chromatids fail to reach the poles. In microspores with more than six chromosomes, certain chromosomes are represented in duplicate. These homologous chromosomes tend to pair, in rare cases, and may even form pseudo-chiasmata. All these various abnormalities are shown in a single microspore in figure 2.

The number of minor coils can be counted in some of the chromosomes. The normal type of metaphase chromosome has about 20 to 25 coils, but some of the long slender chromosomes appeared to have more than 30 coils. These observations, together with similar observations in heat-treated material, indicate that coiling begins with numerous coils of narrow diameter, and that the shortening of the chromosome is effected by a reduction in the number of coils, accompanied by an increase in the diameter of the gyres.

In the triploid the disturbance of chromosome balance causes many abnormalities in development and differentiation of the microspore. The axis of the nuclear division may lie lengthwise of the cell (fig. j) or in a

diagonal position. If both daughter nuclei are formed at the ends of the cell, they may fail to differentiate (fig. k). Frequently the nucleus nearer the wall will be more condensed and will stain darker, but it does not elongate in a normal manner. One or both of the daughter nuclei may divide again (fig. l). Comparatively few pollen grains in the triploid Tradescantia are normally differentiated. We find that abnormalities are also induced by hybridization and X-ray treatment. Apparently genetic factors or small deficiencies, as well as variations in chromosomes balance will inhibit normal differentiation of microspores. The data are shown in table 6.

TABLE 6

Pollen development.

	DEVELOPED—2 OR MORE NUCLEI		UNDEVELOPED	
	DIFFERENTIATED	NOT DIFFERENTIATED	"EMPTY"	TOTAL
T. gigantea (normal)	200 (83%)	18 (7%)	24 (10%)	242
T. gigantea (X-ray)	200 (48%)	64 (15%)	156 (37%)	420
Canaliculata×*paludosa* F_1	200 (46%)	61 (14%)	176 (40%)	435
Triploid *bracteata*	62 (7%)	246 (28%)	565 (65%)	873

The effects of chromosome balance and genetic factors on nuclear differentiation in the microspore are similar to those induced by extreme temperatures (SAX 1935). In a normal microspore the nucleus first lies at one end of the cell; and, as the microspore develops, the nucleus passes to the middle of the cell and near the inner wall of the microspore. A large vacuole is found at each end of the microspore. The nucleus divides across the short axis of the cell. The daughter nucleus near the inner wall does not pass into the typical resting stage, and later elongates to form the crescent-shaped generative nucleus. The other daughter nucleus lies near the center of the cell and becomes a more or less degenerate tube nucleus.

Microspores subjected to high temperatures during development often fail to differentiate normally. The synchronization of nuclear and cytoplasmic activity is disturbed, and the polarity of the cell is changed at the time of nuclear division. As a result, the axis of the nuclear division may lie at any angle in the cell, and the daughter nuclei may not become differentiated into the normal generative and tube nuclei. Additional divisions may also occur in the microspore. These temperature effects are very similar to those induced by chromosome balance in the triploid Tradescantia, and by genetic segregation and X-ray treatment in diploid forms. Apparently both the internal and external agencies which disturb normal differentiation and development have a similar effect on cell de-

velopment. This effect is primarily a change in the rate of chromosome and nuclear development in relation to changes in cytoplasmic organization (SAX 1935, 1937).

Chromosome balance plays an important part in the development of the sporophyte, and many morphological and physiological variations are found in aneuploid segregates of triploids and other unbalanced polyploids. It seems probable that the effects of chromosome balance and genetic constitution on the development of the sporophyte are similar to their effects on the male gametophyte, and that both can be attributed to the disturbance of the normal synchronization of nuclear and cytoplasmic activities.

CONCLUSIONS

Dicentric chromatids at meiosis resulting from crossovers in heterozygous inversions are much more frequent in triploid than in diploid Tradescantias. The difference is attributed to differences in the pairing relationships of the chromosomes in the two forms.

More than half the dicentric chromatids in the triploid are unaccompanied by visible fragments. The absence of visible fragments is attributed to crossovers in short inversions very near the ends of the chromosomes, which release acentric chromatids too small to be seen at meiotic anaphase.

Dicentric chromatids are formed at anaphase of the first postmeiotic division. They appear to be caused by the terminal fusion of sister chromatids following a break in a dicentric chromatid at meiosis.

Variation in chromosome number and chromosome balance in the microspores of the triploid Tradescantia is correlated with (1) the time of nuclear division, (2) the size of the microspore, (3) chromosome length at metaphase, and (4) the disturbance of normal polarity, followed by failure of normal differentiation of the generative and tube nuclei. Failure of normal differentiation of the microspore is also caused by the segregation of genetic factors following hybridization or X-ray treatment, and can be induced by extreme temperatures during microspore development. The various aberrations in chromosome and nuclear development, regardless of their cause, are attributed to the disturbance of the normal synchronization between nuclear and extra-nuclear activities.

LITERATURE CITED

DARLINGTON, C. D., 1929 Chromosome behavior and structural hybridity in the Tradescantiae. J. Genet. **21**: 207–286.

1937 Recent advances in cytology. xvi+671 pp. Philadelphia, P. Blakiston.

KAUFMANN, B. P., 1936 A terminal inversion in *Drosophila ananassae*. Proc. Nat. Acad. Sci. **22**: 591–594.

KING, E., 1933 Chromosome behavior in a triploid Tradescantia. J. Hered. **24**: 253–256.

McCLINTOCK, B., 1932 A correlation of ring-shaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. **18**: 667–681.

1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellf. mikr. Anat. **19**: 191–237.

MÜNTZING, A., 1934 Chromosome fragmentation in a Crepis hybrid. Hereditas **19**: 284–303.

RICHARDSON, M. M., 1936 Structural hybridity in *Lilium Martagon* album×*L. Hansonii*. J. Genet. **32**: 411–450.

SAX, KARL, 1935 The effect of temperature on nuclear differentiation in microspore development. J. Arnold Arb. **16**: 301–310.

1937 Effect of variations in temperature on nuclear and cell division in Tradescantia. Amer. J. Bot. **24**: 218–225.

1937 Chromosome inversions in *Paeony suffruticosa*. Cytologia (in press).

STURTEVANT, A. H. and G. W. BEADLE, 1936 The relations of inversions in the X-chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

UPCOTT, M., 1937 Spontaneous chromosome changes in pollen grains. Nature **139**: 153.

SOME ATTEMPTS TO TEST THE RÔLE OF COSMIC RADIATION IN THE PRODUCTION OF MUTATIONS IN DROSOPHILA MELANOGASTER[1]

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INTRODUCTION

IN 1926 MULLER showed the possibility of producing numerous mutations by irradiating individuals of *Drosophila melanogaster* with X-rays. Since that time the problem has arisen of the extent to which the so-called "spontaneous" mutations found in every thoroughly studied organism may be due to the influence of short wave radiation.

MULLER and other investigators have demonstrated that the terrestrial sources of short wave radiation are far too insignificant to be taken into account in this respect. There remained then only the possibility of the production of mutations by cosmic radiation. Here again MULLER and others have calculated, from the known frequencies of cosmic radiation on earth, that not more than a hundredth of one percent up to a maximum of 3 percent of "spontaneous" mutations in *Drosophila melanogaster* could be attributed to the influence of cosmic radiation. This influence accordingly seemed to be negligible.

But the question arises: to what extent is the basis of all these calculations really safe? They are possible only under the assumption that cosmic rays act upon chromosomes and genes exactly as X-rays do. This assumption seems highly probable but has not been proved definitely. Furthermore, the calculations neglect more or less the formation of secondary radiations from the primary one; and they must so neglect them, since the frequencies of the softer part of the secondary cosmic radiation cannot yet be even guessed by the physicist. This fact has been emphasized recently by RAJEWSKY (1936).

A direct experimental test of the rôle of cosmic radiation in the production of mutations seemed, therefore, highly desirable. For such a test sister cultures from an inbred stock would be exposed to different frequencies of cosmic radiation. The difficulty inherent in such a method is to get sufficiently different conditions.

FRIESEN (1936) in Russia and the author (1936) have tried independently to overcome this difficulty by sending cultures of *Drosophila* up

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into the stratosphere. At the altitude reached by the American stratosphere balloon, the frequencies of cosmic radiation were found to have increased up to 110 times over those at sea level. This advantage was counterbalanced by the shortness of the exposure: the cultures were exposed to these high frequencies for less than four hours. These conditions were even poorer in the case of the Russian stratosphere flight.

No wonder, therefore, that FRIESEN got negative results; while the increase in the mutation rate which I found in the offspring from *Drosophila* cultures taken up into the stratosphere may have been due mainly to the influence of extreme low temperature before and during the flight as well as after the landing (JOLLOS 1936).

There remained another possible way of overcoming the difficulty: to use the differences in cosmic ray frequencies at different altitudes on earth. Physical records taken at an altitude of about 14,000 feet (Pikes Peak, Colorado) have indicated that the frequencies of cosmic radiation at this altitude are about 5 times that at sea level. The disadvantages of such an increase—so small as compared with the stratosphere conditions—could be more than compensated by much longer exposure. This possibility has not been utilized before, as far as I know.

EXPERIMENTAL METHODS

During the summer of 1936 numerous cultures of *Drosophila melanogaster* were kept for various lengths of time on top of Pikes Peak. Sister cultures were kept at Madison, Wisconsin. Two different stocks were used for these experiments: a wild stock ("Oregon") and a spineless stock ("*ss*"). Both had been inbred for over 100 generations. Each culture bottle left on Pikes Peak was wrapped in cotton. A maximum-minimum thermometer was placed with the bottles and all were covered with three inches of cotton and put into a corrugated paper carton filled with fine wood shavings. This box was kept in a heated room of the main building on top of Pikes Peak. Under these conditions the cultures were completely protected against penetration of ultraviolet rays as well as against extreme temperature. During July and August the thermometer inside the box registered between 15° and 24°C; in September between 9° and 23°C. The "normal" mutation rate of Drosophila certainly does not become increased within these ranges of temperature.

Cultures from the Oregon stock were kept on top of Pikes Peak for 5 different periods:

1. July 24–August 17 = 24 days (♂♂ only)
3. July 24–September 2 = 40 days (♂♂, pairs, and their F_1 generation)
3. August 17–September 30 = 44 days (♂♂, pairs and their F_1 generation)
4. September 2–September 30 = 28 days (♂♂ only)

5. July 24–September 30 = 68 days (pairs and their F_1 and F_2 generations)

Cultures of the *ss* stock were exposed for the third and fourth of these periods only.

Only sex-linked mutations were involved in the test, both visible and lethal or semilethal mutations. They were determined by means of mating exposed (or control) males to attached-X or to ClB females.

Previous experiments had shown that four weeks old males of my Oregon and *ss* stocks are able to produce, under normal conditions, sufficient offspring. Even males six weeks old can do so. But, as a rule, they show low fertility, if any. Males remain fertile even for a longer period when kept at a temperature below 24°C. Thus in the tests for the shorter first and fourth periods of exposure it seemed possible to use exclusively males which were exposed to the high altitude conditions in their adult stage only. The results for the second and third period, however, had to be taken partly from males, which were exposed similarly; partly from tests of F_1 males produced in exposed cultures.

The development of a new generation required at least 15 days on Pikes Peak. The cultures which produced the tested F_1 males were started for the second period of exposure on July 24th, each culture with six pairs. The F_1 males from these cultures were transferred to fresh culture bottles on August 17th and tested on September 2nd. They could not, at this time, be more than 25 days old.

The parents of the tested F_1 males from the third period had been kept on Pikes Peak since August 17th. They were transferred to fresh culture bottles on September 2nd. Their F_1 sons, produced in these fresh cultures, were tested on October 2nd and 3rd, at an age of not more than 15 days.[2] These F_1 males and the males exposed in their adult stage only were tested separately. Only slight differences were found between the two groups from the same period of exposure, differences which were statistically negligible. It seemed safe, therefore, to combine in our records the results from both groups.

The males used in the control tests were of a similar age as the corresponding exposed males, thus excluding any influence of age.

THE FREQUENCIES OF VISIBLE SEX-LINKED MUTATIONS

Sufficiently large numbers for a test of *visible* sex-linked mutations have been secured for the third period of exposure only. The results are given in table 1. They include the F_1 males from crosses to attached-X female as well as the F_2 males from ClB tests. In the latter case, of course each F_2 *culture* had to be counted as one, as all males of such a culture carry the same exposed X chromosome.

[2] For period five see p. 539.

TABLE 1

Visible sex-linked mutations.

	NUMBER OF TESTED X CHROMOSOMES	MUTATIONS		TYPE OF MUTATIONS
		NO.	PERCENT	
A. Oregon stock				
Period 3				
Exposed (44 days)	12195	12	0.099±0.0286	5*v*, 3*w*, 2*y*, 2*f*
Controls (from Oct. 10)	11318	2	0.018±0.0125	2*w*
Diff./S.E. Diff.			2.61	
B. *ss* stock				
Period 3				
Exposed (44 days)	9636	13	0.135±0.037	6*v*, 3*w*, 1*w*^{a}, 1*y*, 1*bf*, 1*M*
Controls (from Oct. 10)	10114	2	0.02±0.014	1*w*, 1*y*
Diff./S.E. Diff.			2.88	
A+B				
Exposed	21731	25	0.115±0.023	
Controls	21432	4	0.0187±0.0093	
Diff./S.E. Diff.			3.88	

These records show an increase in the percentage of visible sex-linked mutations in both stocks exposed to the high altitude conditions over 44 days. This increase seems statistically significant, especially if we combine the data from both stocks. We are entitled to do so, as the frequencies of "spontaneous" mutations as well as their increase following exposure are practically the same in both stocks. It should be mentioned that the percentage of visible sex-linked mutations seems unusually high also in the controls.

Table 1 shows another curious result. The mutation "vermilion" was found repeatedly in different cultures and in both independent stocks, but never in the controls. No explanation is offered. It should be emphasized only that the control cultures were examined especially thoroughly for vermilion mutations, since the exposed cultures had been tested first.

The data for the other periods of exposure are too small for a statistical comparison of the frequencies of visible sex-linked mutations. The scarcity of these records was due to an unexpected sterility of the majority of cultures kept on top of Pikes Peak, as discussed later. It should be mentioned that a comparatively high number of visible sex-linked mutations was found also amongst the offspring from cultures from the first and the second periods of exposure. One mutation of this kind (rudimentary) appeared amongst 489 tested X chromosomes exposed over the first period, and two mutations (bifid and again vermilion) amongst 505 tested X chromosomes from the second period of exposure.

THE FREQUENCIES OF LETHAL AND SEMILETHAL SEX-LINKED MUTATIONS

The frequencies of lethal or semilethal sex-linked mutations were determined in four series of Oregon cultures kept on the top of Pikes Peak

over the first, second, third or fourth period, respectively, and in the corresponding controls. Cultures of the *ss* stock were exposed for this test during the third and the fourth periods only. The results are shown in table 2.

Recorded in these tables as "semilethals" are all the cases in which a sex ratio of at least 4 ♀ : 1 ♂ in a minimum of 30 flies was found in the F_2 from ClB crosses (instead of an expected ratio of about 2 ♀ : 1 ♂).

TABLE 2

Lethal and semilethal sex-linked mutations.

A. OREGON STOCK

	NUMBER OF TESTED CHROMOSOMES	LETHALS		SEMILETHALS		LETHALS AND SEMILETHALS	
		NO.	PERCENT	NO.	PERCENT	NO.	PERCENT
Period 1							
Exposed (24 days)	404	6	1.485±0.602	19	4.703±1.05	25	6.188±1.199
Controls (from Aug. 12)	413	none		4	0.969±0.48	4	0.969±0.48
Diff./S.E. Diff.					3.2		4.04
Period 2							
Exposed (40 days)	429	8	1.865±0.65	26	6.06 ±1.15	34	7.925*±1.3
Controls (from Sept. 10)	946	none		13	1.374±0.38	13	1.374 ±0.38
Diff./S.E. Diff.					3.87		4.82
Period 3							
Exposed (44 days)	1291	8	0.620±0.22	37	2.866±0.46	45	3.486*±0.51
Controls (from Oct. 10 and later)	1491	1	0.067±0.067	19	1.274±0.29	20	1.341 ±0.30
Diff./S.E. Diff.			2.419		2.9		3.6
Period 4							
Exposed (28 days)	602	none		11	1.827±0.546		
Controls (from Oct. 10 and later)	1491	1	0.067±0.067	19	1.274±0.29		
Diff./S.E. Diff.					0.89		

B. *ss* STOCK

Period 3							
Exposed (44 days)	605	3	0.496±0.29	20	3.306±0.727	23	3.802±0.78
Controls (from Oct. 10 and later)	663	none		7	1.056±0.39	7	1.056±0.397
Diff./S.E. Diff.					2.7		3.1
Period 4							
Exposed (28 days)	917	none		15	1.636±0.419		
Controls (from Oct. 10 and later)	663	none		7	1.056±0.397		
Diff./S.E. Diff.					1.01		

* For difference between results from periods 2 and 3, Diff./S.E. Diff. = 3.17.

The records show a statistically significant increase in the mutation rate in the offspring of cultures which were exposed to the high altitude conditions for the first, the second, or the third period of our experiments. The results for the fourth period of exposure are negative. This holds true for both stocks (Oregon and *ss*) used in the test. The ineffective exposure in September 1936 lasted even four days longer than the highly effective first exposure in July-August. Furthermore, we find a statistically significant difference (table 2) between the positive results from the second period of exposure and those from the third period. Thus, a 40 days' exposure in July-August 1936 proved to be more effective than a 44 days'

exposure in August-September. An exposure for 24 days in July-August gave distinctly positive results while an exposure for 28 days in September was ineffective.

The data for the first and the second periods are based on a comparatively small number of individuals (404 and 429 respectively). Examinations of larger numbers might have altered the figures a little but hardly to such an extent that it would obliterate the significant difference between the results from July-August and those from September exposures.

THE STERILITY OF DROSOPHILA CULTURES KEPT AT HIGH ALTITUDE

The scarcity of the material available for some of our tests was brought about by the fact that a very high percentage of cultures and individual flies became either completely sterile or significantly reduced in their fertility following prolonged exposure. This alteration was especially pronounced in cultures kept at high altitude for the fifth and longest period, which ran from July 24th to September 30th. Twelve cultures of this series, each containing at the beginning six pairs, produced (to August 17th) a large F_1 generation. On August 17th, 26 F_1 cultures were started, each with six pairs. On August 21st these adult F_1 flies were transferred to a second series of 26 culture bottles. Many F_2 larvae developed within these 52 cultures, but most of them sooner or later became transparent and died. Thus the cultures yielded together up to September 30th, 9 adult flies only, 7 females and 2 males! Out of these, 4 females and both males proved to be completely sterile. This mortality in the larval stage could certainly not be due to old age of the parents. There are indications that it was caused by cytoplasmic disturbances. But the matter needs more extended examination.

DISCUSSION

The records represented in tables 1 and 2 show a significant increase in the percentage of sex-linked mutations in cultures, which were exposed to high altitude conditions for periods of 24, 40 or 44 days in July-August. But they show no appreciable increase in mutation rate following an exposure for 28 days in September.

"High altitude conditions" mean not only higher frequencies of cosmic radiation but also an alteration of some other environmental factors compared with the conditions at sea level (or Wisconsin altitude). We should mention here the differences in temperature, in the frequencies of ultra-violet radiation and in atmospheric pressure and oxygen tension. All these factors may influence the mutation rate; but they cannot be responsible for our results. The cultures were protected against extreme temperature as well as against the penetration of ultra-violet rays, during the whole time of exposure on Pikes Peak. Low atmospheric pressure and

oxygen tension must have been practically the same in July, August and September. They cannot account, therefore, for the positive results from July-August exposures, as the results from September exposures were negative. There seems to be, under these circumstances, no other environmental factor to which the significant increase in the mutation rate following July-August exposures could be due, besides cosmic radiation.

The main purpose of these experiments was to test whether higher frequencies of cosmic radiation increase the frequencies of mutations significantly. The experimental results have answered this question in the affirmative. There was *a significant increase in the mutation rate in both tested stocks following exposures to higher frequencies of cosmic radiation in July-August 1936. This result seems statistically safe.*

There remains, however, the puzzle of the seasonal differences in the results. More actual data are necessary before a definite explanation can be given. We do not know of regular "seasonal" variations in cosmic radiation frequencies which would be significant enough to account for the differences in our results from different periods. Recent observations indicate that this holds true even in regard to the irregular "showers" of cosmic rays.

Furthermore, there was one difference in the setup of the experiments in July-August and that in September, which might give the clue to the puzzle. The cultures were kept, during the whole time, within the same building; but in July and August they were placed directly under a heavy iron structure (tower with staircase), whereas for September they had to be moved, for better temperature control, to a place farther away from metallic surroundings. Physicists agree that passage of cosmic rays through heavy metals leads frequently to outbursts of secondary rays. RAJEWSKY and his collaborators state in a recent publication (1936) that cultures of a fungus showed a significant increase in their mutation rate when kept for a long period inside of metallic chambers which favor the formation of secondary cosmic radiation. One could guess that my Drosphila cultures were kept, in July and August, unintentionally under environmental conditions which favored the formation of secondary rays much more than those in September.

This would mean: (a) that a 28-day exposure to the average frequencies of cosmic radiation at an altitude of 14,000 feet does not increase the frequencies of mutations to an appreciable amount; and (b) that the significant increase in the mutation rate following exposures in July-August was due to the influence of cosmic ray intensities, which were increased much more than 5 times over those at the Madison level.

It should be emphasized that such an interpretation of the actual re-

sults is, at present, an hypothesis only; but it could and should be tested by further experiments.

The data reported in this paper indicate that higher frequencies of cosmic radiation produce a higher mutability. We might conclude, therefore, that the rôle of cosmic radiation in the production of "spontaneous" mutations is not altogether negligible as it has been assumed. But we cannot even guess at the percentage of "spontaneous" mutations due to the influence of cosmic radiation before we know more about the intensities of this radiation which are necessary to produce such positive results as found in our cultures exposed in July-August 1936.

SUMMARY

The mutation rates of two inbred stocks of Drosophila melanogaster, kept for various lengths of time on top of Pikes Peak, Colorado, were tested and compared with those of sister cultures kept at Madison, Wisconsin.

A statistically significant increase in the percentage of visible as well as of lethal and semilethal sex-linked mutations was found following exposure to high altitude conditions for 24, 40 or 44 days in July-August, 1936.

Similar exposure for 28 days in September, 1936, was found ineffective.

The frequencies of cosmic radiation on top of Pikes Peak are, on the average, five times higher than those at Wisconsin altitude. The negative result of September exposures show that the influences of these higher intensities, acting for 28 days, do not produce an appreciable increase in the mutation rate.

The "seasonal" differences in the results may be due to the action of higher frequencies of cosmic radiation in July-August, especially as the cultures were kept during this period in a metallic environment which seems likely to favor outbursts of secondary rays. This hypothetical explanation will be tested by further experiments.

LITERATURE CITED

DELBRÜCK, M., and TIMOFEEFF-RESSOVSKY, N. W., 1936: Cosmic rays and the origin of species. Nature **137**: 358–359.

EPHROIMSON, W. P., 1931: Die transmutierende Wirkung der X-Strahlen und das Problem der genetischen Evolution. Biol. Zbl. **51**: 491–506.

FRIESEN, H., 1936: Die kosmischen Strahlen und der Mutationsprozess. C. R. Acad. Sci. URSS **1**: 183–186.

JOLLOS, V., 1935: Studien zum Evolutionsproblem. Biol. Zbl. **55**: 390–436.

1936: Mutations observed in Drosophila stocks taken up into the stratosphere. Nat. Geog. Soc. Tech. Pap. Stratosphere **2**: 153–157.

MULLER, H. J., and MOTT SMITH, L. M., 1930: Evidence that natural radio-activity is inadequate to explain the frequency of (natural) mutations. Proc. Nat. Adad. Sci. **16**: 270–285.

RAJEVSKY, B., KREBS, A., and ZICKLER, H., 1936 Mutationen durch Hoehenstrahlung. Naturwiss. **24**: 619–620.

THOMAS, H. 1936: Cosmic rays and the origin of species. Nature **137**: 51–53.

TIMOFEEFF-RESSOVSKY, N. W. 1934: The experimental production of mutations. Biol. Rev. **9**: 411–457.

SMALL DEFICIENCIES AND THE PROBLEM OF GENETIC UNITS IN THE GIANT CHROMOSOMES*

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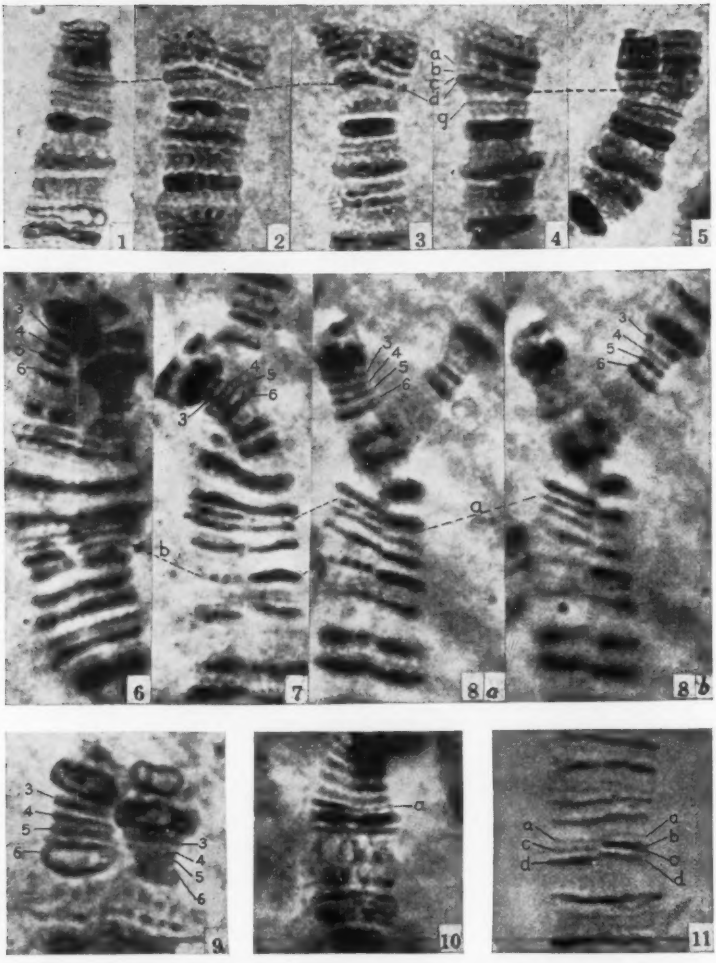
ONE of the main problems involved in studies on the giant gland chromosomes of Diptera is that of visible identification of the genetic units. Two modes of attack on the problem are being followed. On the one hand attention is devoted, through direct observation, to study of structures which look as if they might be units; and on the other, it is devoted, through study of small deficiencies, to structures which behave as units, that is, are acquired or lost as units. The present account deals mainly with the latter aspect, or, more specifically, with two questions which naturally arise in considering this aspect. These are: First, how is it possible to tell when the smallest structure is found which may be acquired or lost as a unit? And second, what is the nature of this structure in terms of the visible chromatic and achromatic constituents in the chromosome?

Since the chromatic material is present in the form of transverse "discs" or "bands" which are readily observed, deficiencies may be identified by detecting "bands" in one homologue which are absent at corresponding loci in the other homologue. One obvious possibility, as suggested by earlier observers, is that a single chromatic band or disc represents a unit locus. But, as all observers have found, the "bands" differ in thickness and many of them are compounds made up of two or more closely approximated discs. This presents a serious difficulty, because at present there is no criterion for determining what is really a single disc. The discs range in thickness down almost to the limits of visibility and it is not known whether the thicker ones merely represent compounds of the thinnest ones, or whether some of them may themselves be really single. This feature will be illustrated in connection with some of the examples considered below.

Further difficulty is presented by the wide range of variation in the structures under consideration, not only from specimen to specimen, but sometimes even in the same gland (METZ 1937 and earlier papers). The variation appears to be due to differences in relative distribution or arrangement of the chromatic and achromatic materials, but its significance is not clear. One possibility is that a considerable amount of achromatic material is normally diffused through the chromatic material of the discs

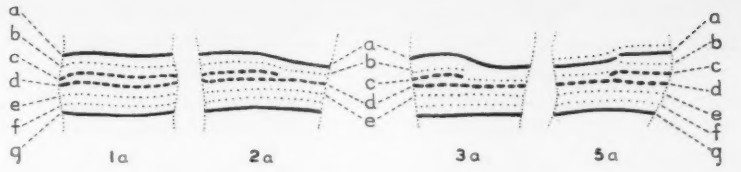
* A part of the cost of the accompanying figures is met by the Galton and Mendel Memorial Fund.



FIGURES 1–11.—Photographs of portions of giant salivary gland chromosomes in *Sciara ocellaris* Comst., illustrating various deficiencies. From aceto-carmine smear preparations. 1–5 and 9 from fresh preparations; the others from permanent mounts made by removing the covers in dioxan and mounting in damar. Magnifications approximately as follows: 1, 1690×; 2–8, 1920×; 9, 2800×; 10, 11, 1920×.

FIGURES 1–5, end 2 of chromosome A illustrating the first example described. 2 and 3 from one specimen; 4 and 5 from another. Compare with the corresponding diagrams in figures 1a, 2a, 3a, 5a.

FIGURES 6–8, end 1 of chromosome A illustrating the fifth and sixth examples described; all from one specimen; 8a and 8b from the same chromosome, at different focal levels. 8b is included



FIGURES 1a, 2a, 3a, and 5a.—Diagrams representing conditions in the corresponding photographs in figures 1, 2, 3 and 5. In 1a bands c and d are present in both homologues; in the others one of them, presumably c, is absent from one homologue. Note that the achromatic zones b–c and d–e on the non-deficient side are like b–d and d–e on the deficient side. In these diagrams bands b, e and f are too light. No significance should be attributed to the number of dots in any of these bands.

and that under different conditions, either in the living state or during fixation, the two separate out in different ways giving different details of pattern. This presumably does not affect the major pattern formed by the linear series of conspicuous discs, but it does complicate the problem of determining what represents a single disc.

The best approach to the present problem as a whole seems to be through comparative study of several small deficiencies, ranging down to the smallest. For this purpose Sciara is particularly favorable because here such deficiencies may readily be found in ordinary stocks without irradiation or other special treatment. Several examples are considered in the present account, which is based entirely on cytological evidence. None of the structures described has yet been identified with any particular genetic character. Some of the deficiencies appear to be common and widely distributed in nature, for they have been found in stocks secured from both North Carolina and Alabama, and at intervals of more than three years. We are indebted to Dr. J. PAUL REYNOLDS for these strains. All the material considered here is from *Sciara ocellaris* Comst.

As will appear below, the general trend of the evidence from these deficiencies seems to indicate that any disc, down at least to the small, thin ones, is divisible into two or more discs sufficiently independent so that one may be lost or acquired without the other. Where the ultimate limit is reached is not yet clear.

FIRST EXAMPLE

The first deficiency to be described is particularly interesting because of the nature of the locus in question and of its immediate surroundings. The

to show the nature of the band at *a* in the non-deficient homologue. This band is partly out of focus in 8a.

FIGURE 9, for comparison with figures 17–20 (see text). Figure 10, portion of chromosome B showing the seventh example described. Figure 11, portion of chromosome B showing the eighth example. For details of all figures see text.

case will be considered in some detail and will be used as a basis of comparison in considering the others. The region is located near end 2 of chromosome A, and the deficiency appears to involve only a single band, as indicated by the photographs shown in figures 1–5. The non-deficient condition is shown in both homologues of the chromosome pair in figure 1, at the point indicated by the dotted line. In this condition the region in question usually exhibits a double "band," or a pair of apparently single bands, flanked on either side by a very thin or light band (at b and e in the accompanying diagrams). Such a grouping is especially favorable for present purposes, as will be indicated below. The double band or pair often appears as shown here, in the form of a wafer-like structure, or narrow "capsule," the two faces of which appear as single bands or rows of granules. In this case the wafer or capsule may enclose a single transverse row of achromatic droplets. In specimens showing the deficiency the non-deficient homologue exhibits the condition just described, while the deficient one lacks one element of the double band or pair, as shown in figures 2–5 and 2a–5a.

Three features of special interest are brought out in this case. (1) The deficiency apparently involves a single chromatic band. (2) The achromatic zones or bands at the left and right of the single chromatic band in the deficient chromosome (above and below in the illustrations) are apparently just like the corresponding ones at the left and right of the double band or pair in the other chromosome. (3) The achromatic material between the two bands of the pair in the latter chromosome appears to be entirely absent from the deficient chromosome. In a descriptive sense at least, the unit difference between the two chromosomes is one chromatic band and the achromatic material between the two bands. So far as visible evidence goes the loss (or acquisition) of a chromatic band here is accompanied by a modification of the achromatic constituent on one side but not on the other. This may be interpreted in two or more ways as noted previously (METZ 1937). It may be assumed that the chromatic disc and the achromatic material on one side of it form a unit. Or it may be assumed that a regulating factor operates to determine the amount of achromatic material between any two given discs. The converse could, of course, be postulated by assuming that the achromatic material determines the nature of the adjacent chromatic discs.

In earlier papers attention has been called to the fact that the achromatic material in these chromosomes is present in the form of droplets giving an alveolar or honeycomb type of organization, modified, of course, by the stratification due to the chromatic discs. Each two successive chromatic discs are separated by a row or transverse plate of achromatic droplets, which may be thick-walled or thin-walled according to the amount of

chromatic material surrounding them. Although not subscribing to this interpretation of chromosome structure, BAUER (1935 and 1936) has called attention to large heavy-walled droplets in Chironomus and evidently considers them as structural units (chromomeres). PAINTER and GRIFFEN (1936) have likewise emphasized such structures in Simulium. The latter authors apparently consider the chromosome as composed of numerous chromonemata each of which includes a series of such droplets ("chromomeres") aligned on a delicate thread. The question of the significance of the droplets and whether they represent parts of chromonemata has been discussed elsewhere (METZ 1937, METZ and LAWRENCE 1937, and earlier papers) and need not be reviewed here except as noted in the discussion.

For present purposes the achromatic material between two successive chromatic discs may be considered as a unit. The immediate problem is that of ascertaining the actual relationship between this material and the adjacent chromatic material. Several possible interpretations suggest themselves. These differ largely in the relative significance attributed to the chromatic and achromatic materials. If the chromatic discs represent the genic material and the achromatic interspaces are merely composed of non-genic connecting material, then attention may be confined mainly to the former, and the significant unit is the disc. If, however, the achromatic material is considered significant, the essential unit should be achromatic or should be represented by a combination of achromatic and chromatic materials. It is difficult to consider the unit as asymmetrical and composed of a chromatic disc combined with the achromatic material on one side of it. This would presumably require a regular orientation of all units, which is opposed by the evidence from inversions. An alternative interpretation would be that chromatic and achromatic materials are combined to form a unit, but that such a unit consists of three layers instead of two. It could consist of a chromatic disc lying between two layers of achromatic material, or of a layer of achromatic material lying between two layers of chromatic material. In either case the unit would be a wafer-like structure in which the two faces differed from the interior.

Several lines of evidence suggest that the giant chromosome might be made up of such wafer-like units in the manner indicated schematically in figure 12. We have attempted to apply hypotheses of this kind but have not yet found one which shows a satisfactory agreement with the evidence. Brief attention may be given, however, to one such hypothesis which appears to come closest to fulfilling the requirements at present. It is the last one mentioned above, illustrated in figure 12.

On this hypothesis the chromosome may be considered to be composed of a series of closely appressed wafer-like segments, each segment composed of a plate of achromatic material or droplets, the two faces of which

are covered by a layer of chromatin. On this view a chromatic disc would represent the interface between two closely appressed segments and be composed of the chromatin of these two appressed faces. On such an interpretation a single segment deficiency should not involve the entire loss of a single chromatic disc but rather the loss of an achromatic plate together with part of each of the two adjacent discs. A break in the chromosome, therefore, should occur within a chromatic disc rather than between discs. It would be reasonable to assume on this view that the two faces of the hypothetical segment should be alike. Two lines of evidence should be particularly important in testing this hypothesis. It would be expected, on the one hand, that heavy bands or discs would not appear

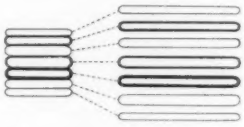


FIGURE 12.—Diagram illustrating one hypothesis of chromosome structure considered in the text This hypothesis is not supported by the evidence.

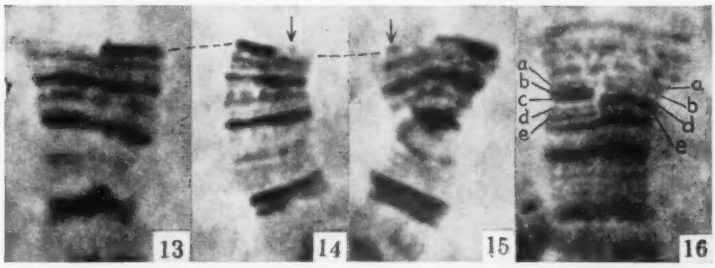
singly, because if one face of the segment is heavy the other one should likewise be heavy. Unfortunately, the evidence on this point is not conclusive because, although apparently single heavy bands may be observed, it is not yet possible to make certain that they are actually single.

The second line of evidence is that provided by cases such as the one described above. It involves a deficiency in a region having a moderately heavy double band or pair flanked on either side by very light bands. The two bands of the pair should represent the two faces of one wafer or segment. If this segment is lost, the two bands should disappear and leave one light band representing the faces of the two adjoining segments. Apparently, however, this does not occur, for the remaining band (d, in figures 2a, 3a, 5a and photographs) is heavier than would be expected on this basis, and appears to correspond to its apparent mate in the other chromosome. To explain the results on the hypothesis under consideration, therefore, would require the assumption that the two faces of the hypothetical segment are not alike, and there seems no legitimate ground for making this assumption at present. The evidence seems best interpreted on the assumption that the chromatic disc is lost or acquired as a unit, unless we assume that the two component discs under consideration are not single. If each of them is itself a pair, then of course the situation is entirely different; but, as will be shown below, deficiencies of this general type are found which involve the loss or acquisition of "single" delicate bands that are almost as small as the smallest which can be detected. The

evidence is strong that they are really single and require the interpretation just given.

SECOND EXAMPLE

The second case is interesting in connection with that just considered because it apparently involves the same general type of original structure, but a different type of loss. It is a terminal deficiency, at end 2 of the same chromosome. In the non-deficient condition (figure 1) this chromosome terminates in a structure which may appear as a single heavy band, a double band, a pair of bands, or a small "capsule" inclosing a single transverse row of achromatic droplets. The deficiency in the present case, however, involves the loss of both members of the pair instead of only one, as indicated in figures 13–15. These figures are all from one specimen. The glands were fixed in formalin, followed by 45 percent acetic acid, and stained in crystal violet.



FIGURES 13–16.—Photographs illustrating the second and fourth deficiencies described in the text. 13–15 from a formalin-acetic-crystal violet preparation, 16 from the same preparation as figures 6–8. All approximately 2350×. 13–15, end 2 of chromosome A; 16, end 2 of the X chromosome. See text for details.

In the present case it is not clear as to just what is lost in addition to the pair of chromatic bands. At first sight it looks as if the adjacent achromatic material was not lost, and that the deficient homologue terminates in a zone of achromatic material. This is suggested especially by figure 13. It seems probable, however, that this is not the case, and that the terminus here is a very delicate chromatic band which is not visible in the non-deficient chromosome. Such a band is evident in this position in some preparations where no terminal deficiency is involved.

Certain characteristics of this delicate terminal band in the deficient homologue should be noted at this point because of their bearing on the question as to the significance of the chromatic "granules" in the giant chromosomes. In some cases, such as those shown in figures 14 and 15, most of the chromatin in this delicate band, or disc, has apparently ag-

gregated into one or two small lumps or "granules" (indicated by arrows). These show the same characteristics as the "granules" in other regions, yet it seems clear that they cannot represent individual genes in accordance with the interpretation advanced by some authors (for discussion see METZ 1937, METZ and LAWRENCE 1937).

THIRD EXAMPLE

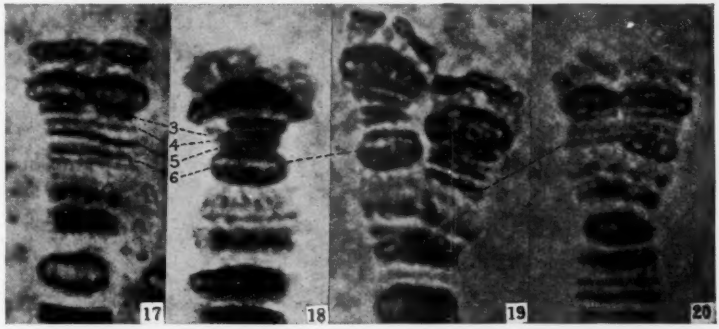
A third deficiency in this same chromosome appears to present a combination of the characteristics seen in the other two. In the non-deficient condition the structure under consideration apparently includes two similar double bands, which usually combine to form a large capsule; and the "loss" apparently involves an entire double element. The region is near end 1 of chromosome A, at the locus marked 6 in previous illustrations (METZ 1935a, figures 4 and 5). This region has been studied in hundreds of cells, including many examples of each of the three possible types, homozygous non-deficient, homozygous deficient and heterozygous. It is not known which condition is to be considered typical for the species. Many specimens exhibit at this locus what appears to be a single band or row of granules, which often forms a wafer-like structure or slender "capsule." In the latter case the single band or row of granules becomes transformed into a double band or two rows of granules, as indicated at 6 in figure 17 (also METZ 1935, figure 5, C). This condition has been discussed in another paper (METZ and LAWRENCE 1937). The adjacent band 5, which is typically a narrower band and often definitely a single row of granules (figures 17, 18, 20 and METZ 1935, figure 5, A, B) shows these same characteristics.

In these cases the differences in condition apparently reflect differences in the distribution of the achromatic in relation to the chromatic material. The "single" disc apparently becomes "double" by acquisition of achromatic material within it. In the resulting wafer-like structure each chromatic face looks like a disc. Both conditions may be found in one gland.

The structure just described, whether designated a single band, a double band or a pair of bands, evidently represents the homozygous deficient condition. In contrast, other specimens exhibit at locus 6 a much larger, heavy "capsule," as shown in figure 18, or two double bands each of which is as heavy as that just considered. This represents the homozygous non-deficient condition. Unfortunately, the strong tendency of this structure to form a capsule makes it very difficult to study the finer details of organization. It seems clear that the structure involves two relatively heavy double bands and intervening achromatic materials, as just indicated; but whether or not it also includes more than this is not certain. Apparently it does not.

The heterozygous condition is represented in figures 9, 19 and 20. As would be expected, the structures in the deficient and the non-deficient homologues correspond respectively to those just considered. The main characteristics are made clear by the fact that the structure on the deficient side may appear either as a single band, as in figure 20, or as two light bands or rows of granules, representing the two faces of the "wafer" or "capsule," as in figure 9. These two conditions may be found within the same gland.

It seems evident that the loss or acquisition here involves one double band or wafer, together with the achromatic material between the two.



FIGURES 17–20.—Photographs illustrating the third deficiency described. All represent end 1 of chromosome A; from fresh aceto-carmine preparations. 2500×. See also figure 9. 17 has been published previously (METZ 1935, Fig. 5 D). The numbering of the bands corresponds to that in figures 6–9 and in previous publications. In some cases, such as shown in figure 17, a narrow band, not numbered, is seen between 5 and 6, close to 6. For details see text.

As in the first example considered there is no apparent effect on the achromatic material to the left and right of the bands under consideration. The achromatic zones adjacent to the structure on the deficient side appear to be just like those adjacent to the larger, double structure on the non-deficient side.

In superficial aspects the present deficiency resembles the first example described, but actually the structures involved here all appear to be double as compared with those in the other case. In other words, the first example appears to represent the loss of one component of a double band, that is the loss of a single band; whereas in the present case it represents a loss of an entire double band.

FOURTH EXAMPLE

This example involves a deficiency of the type seen in the first example, but the size of the structures is more like that of those in the second case

described. The region is located near end 2 of the X chromosome, as indicated in figure 16. On the non-deficient side there is a conspicuous double band, or pair (bc) while in the deficient chromosome, on the other side, there is a single band at the corresponding locus (b). In this particular nucleus, at this focal level, the two adjacent bands d and e are not continuous across the chromosome, and their appearance suggests the possibility of an inversion. This is not the case, however, as shown by other nuclei, where they are continuous.

Aside from the fact that this example exhibits characteristics in conformity with those already discussed, it is of interest because it may possibly involve loci responsible for sex determination. As indicated in earlier papers (METZ 1931, METZ and SCHMUCK 1931) there are two kinds of X chromosomes in *Sciara coprophila*, one designated X and the other X-prime (X′). Females homozygous for X are male-producers, while those carrying X′ are female-producers. Comparable conditions appear to exist in the present species, and the widespread occurrence of the chromosome condition described and figured here suggests that it may represent the X′X condition. This possibility is being investigated.

FIFTH EXAMPLE

This example is essentially like the last and needs little description. It is shown in figures 6–8 at the locus marked *b*. The deficient homologue possesses a narrow band (fig. 6) or row of granules (figs. 7 and 8a), while the non-deficient one possesses a double band apparently composed of two of the single ones. This case is included because it accompanies a smaller deficiency, next to be described. The two are found near end 1 of chromosome A, the former at the point designated 13 and the latter between points 10 and 11 in previously published photographs (METZ 1935, figure 5).

SIXTH EXAMPLE

This case possesses special interest because of the small size of the structures involved. They are only about half the size of the smallest ones considered above, as shown at a in figures 7 and 8. The band on the non-deficient side, shown at the left in the figures, usually appears single. But it is clearly double in one cell of the specimen from which these photographs were taken. It presumably represents two closely approximated, very narrow discs. On the opposite side only a single dotted line appears, which is often so faint as to be barely visible. Apparently it represents one of the two delicate discs going to make up the band in the other homologue, for it connects with the double structure and shows the ordinary indications of homology.

This deficiency involves a structure almost as small as any which can be examined satisfactorily, and it seems highly probable that it represents a "single" disc if this term has any significance in more than a descriptive sense.

SEVENTH EXAMPLE

Like the case just described, this one involves very small structures. It is found a short distance from end 1 of chromosome B, and is represented at a in the photograph shown in figure 10.

The deficient homologue, at the right, shows a delicate band or row of delicate granules, and the non-deficient member shows at the same locus a narrow band which apparently represents two delicate discs, one of which is presumably the counterpart of the single one on the other side. Here again the difference involves a disc about as small as any which could be used with confidence in such a study.

EIGHTH EXAMPLE

The present case is essentially like the two preceding ones, but the structures are somewhat larger, as shown in figure 11, at bc. The band at d is continuous across the chromosome in other cells, showing that the condition does not represent an inversion. This deficiency also is found near end 1 of chromosome B, but is closer to the end than is the preceding one.

Discussion

Small deficiencies visible in the salivary gland chromosomes have been described by several investigators, including MACKENSEN (1935), MULLER (1935), MULLER and PROKOFIEVA (1935), DEMEREC and HOOVER (1936) and EMMENS (1937), but so far as I am aware no detailed evidence has been presented bearing on the particular aspect under consideration in the present paper. Two of the three drawings shown by EMMENS to illustrate the deficiency known as "roughest²" (his fig. 3) in *Drosophila melanogaster* represent a large achromatic zone in the deficient chromosome opposite the two prominent bands in its mate. Taken alone, these would suggest that in this case loss of chromatic bands was not accompanied by loss of achromatic material. But in the third drawing no achromatic zone is shown at the point of deficiency; the gap is completely closed. This fact, together with the author's statement that the chromosome is usually buckled at this point and that good figures are difficult to secure, makes it probable that the gap in the first two figures is due to stretching the deficient homologue and that the actual condition agrees in this respect with that described in the present paper.

The present account is mainly concerned with three questions: (1) What is the smallest unit which can be detected through study of small defi-

ciencies in the giant chromosomes? (2) What is the composition of this unit in terms of the visible chromatic discs and achromatic materials? (3) What light do the results of such a study throw on the nature and relationships of the chromatic discs and the achromatic materials?

Eight deficiencies have been described, ranging from cases involving two or more discs, down to others involving apparently single, very small discs. The latter appear to be almost as small as the smallest detectable discs. The study is not concerned with the genetic effects of any of the structures, but only with morphological aspects of organization.

Each of the "single band" deficiencies represents the loss or acquisition of one element of a double band or pair of bands. Whether or not the two are alike, or duplicates, in more than a morphological sense, is not known, but, as noted below, it seems probable that they are. The "unit" in each of these cases includes what appears to be a single disc, and also, in a descriptive sense at least, the achromatic material between the two similar discs. The loss or acquisition of a disc is apparently accompanied by loss or acquisition of achromatic material; but whether or not the two materials are actually combined in a definite manner to make a unit is not clear. This question is discussed in some detail in connection with the first example described.

The bearing of these findings on the interrelationships of chromatic and achromatic materials, and on the nature of the discs, is also discussed to some extent in the section just referred to. There are certain additional features, however, which may be noted in this connection. Attention has been called by several investigators, including BRIDGES (1935), BAUER (1936) and PAINTER and GRIFFEN (1936), to double, scalloped bands or to pairs of bands which may in some cases appear as single rows of conspicuous, heavy walled droplets or as wafer-like structures of the type described above. These droplets have been considered as discrete structural units, or chromomeres capable of reproduction, especially by BAUER and by PAINTER and GRIFFEN. On the view of PAINTER and GRIFFEN the pair of bands (discs) and intervening material represent a transverse plate of chromomeres and hence a unit locus. The structure should, therefore, behave as a unit; neither disc could be lost or acquired without the other. These authors apparently apply this conception to double bands in general, including the type considered in the present paper.

The cases described above involving a difference of one band of a pair provide strong indication that double bands or pairs do not represent units, and that consequently the single row of droplets, often visible in these cases, is not made up of unit chromomeres. This interpretation is given weight not only by conditions in the individual cases, but by the fact that they all agree, and that the range extends down to such small bands.

So far as the evidence goes it seems to indicate that each detectable disc is essentially independent, in the sense of being separable from its neighbors. If this is true it is difficult to attribute significance as genetic units to the achromatic droplets, either thick-walled or thin-walled. This agrees with the inferences drawn from other characteristics exhibited by these droplets, as indicated in earlier papers cited above.

The question might be raised as to whether or not, in the cases treated here, the single band in the deficient homologue is really homologous to one element of the double band in the other homologue. If it were not, the deficiency in each of these cases would be a deficiency of two discs instead of one, and the interpretation might be distinctly different. The evidence seems clear on this point, however, and seems to show that the homology exists, as described. This is indicated not only by the continuity of the structure across the double chromosome in many cases, but also by the similarity of conditions in the different cases.

The fact that in each case of a "single band" deficiency the single disc on the deficient side is matched by two similar discs on the non-deficient side suggests strongly that the cases involve duplication (acquisition) of a disc, rather than loss. This tends further to support the interpretation of homology just mentioned.

The evidence from these deficiencies agrees with that from most studies on the giant chromosomes in pointing toward the chromatic rather than the achromatic materials as the genetically important constituents. Both may exhibit considerable variation in appearance, but apparently the variation in the chromatic discs is largely due to variations in the amount and distribution of achromatic materials.

SUMMARY

Eight small deficiencies are described (in Sciara) in which one homologue possesses structures not present at the corresponding locus in the other.

Two involve the loss or acquisition of a double chromatic disc or pair of discs; the others involve only one member of such a double structure. The smallest ones are so small as to make it highly probable that they represent really single discs.

In descriptive terms, the smallest unit loss or acquisition involves one chromatic disc and the layer of achromatic material on one side of it, but it seems improbable that the unit is really made up in this manner.

The bearing of the findings on some of the current hypotheses of chromosome organization is discussed, and it is concluded that the heavy walled droplets, described as representing unit genes or chromomeres by some authors, probably are not unit structures and do not represent unit loci.

LITERATURE CITED

BAUER, H., 1935 Der Aufbau der Chromosomen aus den Speicheldrüsen von *Chironomus Thummi* Kiefer Z. Zellf. mik. Anat. **23**: 280–313.

1936 Beitrage zur vergleichenden Morphologie der Speicheldrüsenchromosomen. Zool. Jahrb. **56**: 239–276.

BRIDGES, C. B., 1935 The structure of salivary chromosomes and the relation of the banding to the genes. Amer. Nat. **69**: 59.

EMMENS, C. W., 1937 Salivary gland cytology of Roughest^3 inversion and reinversion, and roughest^2. J. Genet. **34**: 191–202.

METZ, C. W. 1931 Unisexual progenies and sex determination in Sciara. Quart. Rev. Biol. **6**: 306–312.

1935 Structure of the salivary gland chromosomes in Sciara. J. Hered. **26**: 177–188.

1937 Deficiencies and structural variations within the giant chromosomes in relation to the problem of gene structure. Proc. Nat. Acad. Sci. **23**: 137–142.

METZ, CHARLES W., and SCHMUCK, M. LOUISE, 1931 Studies on sex determination and the sex chromosome mechanism in Sciara. Genetics **16**: 225–253.

METZ, CHARLES W., and LAWRENCE, ELIZABETH GAY, 1937 Studies on the organization of the giant gland chromosomes of Diptera. Quart. Rev. Biol. **12**: 135–151.

MACKENSEN, OTTO, 1935 Locating genes on salivary chromosomes. J. Hered.—**26**: 163–174.

MULLER, H. J., 1935 A viable two-gene deficiency. J. Hered. **26**: 469–478.

MULLER, H. J., and PROKOFIEVA, A., 1935. The individual gene in relation to the chromomere and the chromosome. Proc. Nat. Acad. Sci. **21**: 16–26.

PAINTER, T. S., and GRIFFEN, ALLEN B., 1936. The origin and structure of the salivary gland chromosomes of *Simulium vergatum*. Records of the Genetics Society of America **5**: 202–203.

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STANFORD, ERNEST ELWOOD. *General and economic botany.* Pp. XXIX + 675, figs., 436. D. Appleton-Century Co., New York. May 21, 1937. $4.00.

Work of the Mountain Talga Station of the Far Eastern Branch of the Academy of Sciences, U.S.S.R. (Trudi—Gornotakzhnoia Stantsii Dal'nevosto nogo Filiala Akademii Nauk SSSR) Vol. I. Pp. 319, fig. 1. Vladivostock, U.S.S.R. 1936.

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